EXPRESS MAIL MAILING LABEL
NO. EV399913525US

PATENT
Atty. Docket No. CWP-012CN
(1451/2)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Charlton et al.		
SERIAL NUMBER:	08/465,675	GROUP NUMBER:	1817
FILING DATE:	June 6, 1995	EXAMINER:	C. A. Spiegel
TITLE:	TEST DEVICE AND METHOD FOR COLORED PARTICLE IMMUNOASSAY		

CERTIFICATE OF HAND DELIVERY

I hereby certify that this correspondence is being delivered by hand to Examiner C.A. Spiegel at the U.S. Patent and Trademark Office on October 15, 1997.

10/14/97
Date of Signature

Assistant Commissioner of Patents
Washington, D.C. 20231

SHOWING UNDER 37 CFR 1.608(b)

Sir:

In still further response to the Office Action dated August 7, 1997, and as requested by the Examiner in charge of this application, Applicant hereby submits a showing pursuant to 37 C.F.R. 1.608(b) that the subject matter of the proposed count was in his possession prior to

March 27, 1987, the filing date of U.S. Patent No. 5,591,645 to Rosenstein, and therefore demonstrates that Applicant is *prima facie* entitled to judgment relative to the Rosenstein patentee. This submission is of the form consistent with the letter and spirit of the law articulated in 37 CFR 1.608(b) and MPEP §2308 and cases cited therein. This submission includes four Declarations and a detailed explanation which together satisfy the requirements of 37 CFR 1.608(b). More specifically, this submission includes the declarations of 1) David E. Charlton, the inventor of the subject matter which falls within the count, 2) Neil W. Miller, a named inventor on this application, but not properly a co-inventor of the subject matter claimed in this application and which falls within the count, 3) Margaret Mazzeo (née Margaret Verbanic), a scientist working under Dr. Charlton's direction at the time of actual reduction to practice of the subject matter of the count, and a corroborating witness, and 4) Kevin B. Clarke, patent counsel to the assignee of this application, Carter-Wallace, Inc., who authenticates various documents and sets forth the factual basis for their admissibility into evidence under the Federal Rules of Evidence. The submission also includes Exhibits A through M, forming an integral part of the 608(b) showing. The required explanation, stating with particularity the basis upon which the applicant is *prima facie* entitled to judgment, is set forth below.

Also enclosed is a Petition and Amendment for Change of Inventorship under 37 CFR 1.48(b) along with the required fee and a joint declaration of Charlton and Miller indicating that Neil Miller is not an inventor of the claimed subject matter as it is now pending in this application as a result of the amendment submitted to the Patent Office on April 7, 1997 and modified by the Supplemental Amendment submitted on September 4, 1997.

REMARKS

The Examiner-in-Chief should hold that applicant is *prima facie* entitled to judgment, as this submission demonstrates that before the Rosenstein Patent's March 27, 1987 filing date, the applicant actually reduced to practice in the United States the subject matter of the count, at least four separate times. More particularly, the submission shows applicant's actual reduction to

practice of the subject matter of both the first alternate section of the count, relating to a test device which operates in the sandwich format, and the second alternate section of the count, which relates to a method of detecting an analyte using the test device in the sandwich format.

Broadly, Dr. Charlton, a named inventor on the application and the sole inventor of the subject matter of the count, explains in detail in his declaration how each of the notebook pages attached as Exhibits A, E, F, and G evidences a separate instance of actual reduction to practice of the subject matter of the first and second parts of the four part count (separated by "or"s). Dr. Charlton's declaration also refers to Exhibits H, I, J, K, L, and M, which further collectively corroborate the making of his invention. In particular, Exhibits H, I, and J are memoranda authored by an employee of Carter-Wallace at the time relevant here, and describe , in summary fashion, work conducted by Dr. Charlton, through his technical assistant Ms. Mazzeo, née Verbanic. The Memoranda were prepared and circulated shortly after Dr. Charlton and his assistant performed the work referred to therein. Exhibits K and L are memoranda authored by Dr. Charlton which provide additional documentary support corroborating the actual reduction to practice of his invention. These memoranda have been present in the files of Carter-Wallace from a time prior to March 27, 1987 as declared by Mr. Clarke. Exhibit M is a copy of US patent serial no. 4,313,734 to Leuvering which discloses methods of making gold conjugates, a component of Dr. Charlton's invention used by Ms. Mazzeo in the test devices shown in Exhibits A, E, F, and G. Margaret Mazzeo's declaration corroborates the actual reduction to practice demonstrated and evidenced by Dr. Charlton with respect to Exhibits A, E, F, and G. Ms. Mazzeo is the author of notebook pages identified as Exhibits A through G. She is not an inventor here, but was the person who did the hands-on laboratory work under Dr. Charlton's direction. Accordingly, her detailed explanation of work she did under Dr. Charlton's direction constituting a reduction to practice of the subject matter of the count serves two purposes: 1) to evidence facts and acts done that amount to an actual reduction to practice of Dr. Charlton's conception of devices and methods that satisfy the first and second alternate sections of the count; and 2) to corroborate the actual reduction to practice.

In addition to the color photostatic notebook pages marked as Exhibits A, E, F, and G, Ms. Mazzeo, née Verbanic, in her declaration, also discusses notebook pages attached to her declaration as Exhibits B, C, and D. Exhibit B evidences Ms. Mazzeo's preparation in the laboratory of antibody coated latex, and use of that preparation to construct a test device including strips of permeable material each having a site for visually determining the presence of colored particles formed by immobilizing the sensitized latex preparation in a portion of the permeable material. Exhibit C evidences the preparation in Dr. Charlton's laboratory by Ms. Mazzeo, née Verbanic, of antibody labeled gold particles of approximately 15 nm in diameter. This reagent was used in an actual reduction to practice to supply the "binder for the analyte coupled to a colored particle" required by the count. Such conjugates were known in the art prior to the making of the invention as is apparent from the disclosure of this patent application at page 15, ¶ 2. Exhibit D is a notebook page written by Ms. Mazzeo, née Verbanic, recording 1) the procedure followed to apply antibody-coated latex onto a portion of each strip, and 2) a test demonstrating that the latex beads in fact were immobilized on the strip material used as a permeable material in the reduction to practice of the invention. Ms. Mazzeo, née Verbanic, also authenticates Exhibit J, and, further corroborating the actual reduction to practice of Dr. Charlton's invention, declares that she attended the meeting referred to in the memo and received a copy of the memo on or around the time of its date.

The Declaration of Neil W. Miller, a co-inventor named on the above-referenced application, explains 1) that it was Dr. Charlton who conceived of the easy assay "dipstick" and "pee-on" formats that are the subject of the reduction to practice, 2) that Dr. Charlton informed Mr. Miller of these conceived test devices and test procedures, and 3) that Mr. Miller's role in this work was in developing together with Dr. Charlton specially designed housings or casings for the test strips. As stated in the joint declaration of Dr. Charlton and Mr. Miller submitted herewith, Mr. Miller is not a co-inventor of the subject matter now claimed in this application because neither the test device nor the test method of the count requires a housing, and no claims now

pending in the application recite a housing. Mr. Miller's declaration therefore serves to corroborate further the making of the invention by Dr. Charlton.

Mr. Kevin B. Clarke's Declaration established that all of the documentary exhibits, except for Exhibit M, included herewith are admissible in evidence as corporate records kept in the ordinary course of business. Mr. Clarke's declaration also corroborates Dr. Charlton's assertion that he invented the subject matter of the count before the Rosenstein Patent filing date, in that Mr. Clarke declares that the documents he obtained from the files of Carter-Wallace, Inc. had been in the files of Carter-Wallace, Inc. from a time prior to March 27, 1987. Accordingly, the documents themselves corroborate Dr. Charlton's statements.

DETAILED EXPLANATION

1. Actual Reductions to Practice

Turning now to the substance of the showing, Dr. Charlton, corroborated by the declaration of Ms. Mazzeo, née Verbanic, has established that each of Exhibits A, E, F, and G records experiments which constitute actual reductions to practice of the first and second sections of the count and which were conducted prior to the Rosenstein Patent's filing date. These Exhibits show color photostatic copies of the actual test devices useful for the determination of the presence of hCG which were fabricated by Ms. Mazzeo. Further, these Exhibits are records of assays conducted by Ms. Mazzeo using the fabricated test devices to determine the presence of hCG in liquid samples.

As explained in detail in the declarations of Ms Mazzeo, pages 4-8, ¶ 18-24 and of Dr. Charlton, pages 3-5, ¶ 12-15, Exhibit A discloses the use of 1) four glass fiber strips on a "gel bond support" (and two nitrocellulose supports) to "Test Feasibility of Chromatograph Assay," 2) 15 nm average size gold particles conjugated to antibody (B108), and 3) latex particles having antibody (151) attached thereto. Both B108 and 151 are binders (antibodies) for hCG.
Ms. Mazzeo declares that the sensitized latex had been used in then-existing Carter-Wallace

pregnancy test kits. At page 6, ¶ 21, she explains that she performed the described experiment by contacting the bottom most portion of the test strips into the liquid samples she had prepared (urine samples containing known amounts of hCG and gold conjugate), whereby the liquid, and with it the conjugate, wicked up the strip, and, as a result, a "sandwich" (gold antibody conjugate-hCG-immobilized latex antibody to hCG) became immobilized at the test site. The agglomeration at the test site of the gold particles permitted Ms. Mazzeo to observe directly that hCG was present in certain of the test samples, and to determine that there were varying concentrations of hCG which could be correlated with depth of color.

Dr. Charlton and Ms. Mazzeo explain and document, with respect to Exhibit E, a second, separate reduction to practice of the subject matter of the count. As explained in Ms. Mazzeo's declaration, pages 8-11, ¶ 27-31, after making test batches of reagents using the generally established protocols as set forth on Exhibits B and C, and immobilizing antibody at a spot on the test strip using a technique of the type generally disclosed in Exhibit D, Ms. Mazzeo used dilutions of hCG standard solutions having known hCG concentrations (including hCG-free solutions) as test samples, and mixed them together with the gold conjugate (pages 11-13, ¶ 32-34 of Ms. Mazzeo's Declaration). Here, the 151-sensitized latex again was used on glass fiber paper, but the inventor and his laboratory assistant were experimenting to investigate the effect of the use of different sized gold particles. Ms. Mazzeo describes the use of a 30 nm diameter gold particle in addition to the 15 nm gold particle previously used, both lyophilized in a buffer. The ends of the various strips of glass fiber paper which, downstream, included applied latex test spots (immobilized antibody), were contacted with liquid samples of known hCG concentration mixed with 15 or 30 nm gold conjugate prepared with a urine pool. The results of these experiments are shown at the bottom of Exhibit E where the strips were affixed to the paper. Again, it is manifest that Dr. Charlton and Ms. Mazzeo successfully observed passage along the strip of sample together with reconstituted conjugate as the liquid was wicked up the strip. They also observed development of color at the test site. Thus, as soon as the liquid sample was applied on the bottom portion of the strip and as the liquid sample wicked up the strip, the conjugate became

movably supported within the first portion as required by the count. To this day, the strips, and indeed the photostatic copy of the original, permit one to distinguish visually between, for example, a solution containing 1 IU hCG and an hCG-free urine.

With reference to Exhibit F, Dr. Charlton and Ms. Mazzeo explain still another reduction to practice of the subject matter of the first and second sections of the count. In Exhibit F, conjugate (gold 15/b108 or gold 30/b108) was deposited on Whatman™ paper discs, and both the discs and the permeable material used for the assay were fixed to a backing. Sample was then applied onto the disc and color (conjugate) left the discs and developed at the test site as the sample entrained the conjugate, moved up the strip, and conjugates accumulated at the test site. Exhibit F, like the others, contains the actual devices used in the experiment affixed directly to the page. Exhibit F also includes disposed above each of the test devices discs (one missing) of the various types of Whatman™ paper before application of the liquid sample. Again, from a comparison of colors between the disc pairs and the clearly visually detectable test spot on the strips, it is manifest that sample, together with reconstituted conjugate, passed through the test site where the conjugate was captured.

Ms. Mazzeo and Dr. Charlton describe still another, separate, reduction to practice before the Rosenstein Patent filing with reference to Exhibit G. This experiment was done using glass fiber paper as a permeable material, 30 nm gold conjugate with antibody to hCG (B108), and antibody 151 sensitized latex particles. As explained by Ms. Mazzeo and Dr. Charlton, this experiment was designed to determine the maximum concentration of hCG that could be detected using the test device and method which Dr. Charlton had developed with the aid of Ms. Mazzeo. Again still visually detectable color was developed in all test where hCG was present in the urine samples, except that very high concentration of hCG (100 IU), as predicted by Dr. Charlton, resulted in no detectable color development, as uncomplexed hCG competed successfully with hCG complexed with gold for binding at the test site.

2. Comparison of Actual Reductions to Practice With the First Two Sections of the Count

In each of the four experiments referenced above, and shown in Exhibits A, E, F, and G, Dr. Charlton, through the efforts of Ms. Mazzeo, constructed a test device for determining an analyte in a liquid sample, specifically, in all of these cases, hCG. The devices comprised a strip of glass fiber paper or, in a case of Exhibit F, glass fiber paper and a "Whatman™ paper", or in the case of the second experiment in Exhibit A, cellulose acetate paper, all of which are permeable materials as required by the first two alternate sections of the count. The present specification and the Rosenstein Patent disclose these materials as being useful strip materials. Permeability is understood to be required to enable transport of liquids along a paper by chromatography or capillary flow.

In all cases discussed herein, shown in Exhibits A, E, F, and G, the permeable material used in the devices can be seen as defining a first portion and a second portion, as required by the first two alternate sections of the count. The first portion is the bottom most portion of the strips in Exhibit A, E, and G and the top most portion of the strip, including the discs of Whatman™ paper, in Exhibit F. In the devices used for assays shown in Exhibits A, E, and G, the site of application of the liquid samples did not exceed the 1/4 inch bottom most portion of the strips as the strips were placed in test tubes of 10x50 mm size containing 250 µl of liquid sample (see page 12, ¶ 32 of Ms. Mazzeo's Declaration). The second portion is the area on the strip to which the antibody was immobilized via the latex particles which stick to the permeable fiber paper. The second portion is distinct from the first portion as it is located about 1/2 inch up from the bottom most portion of the strips, which is about 1/4 inch above the first portion. Both portions are in the same plane and permit (in fact actually induced) capillary flow communication with each other as also required by the first two alternate sections of the count. With respect to Exhibits A, E, and G, it is manifest that the first and second portions are in the same plane as they are made of a single strip. With respect to Exhibit F, the discs and the adjoining strips were stuck to the same piece of a backing material, i.e., gel bond, thus having one of their surface facing the backing in

the same plane. That the devices "permit capillary flow communication" between the first and second portion is manifest from the evidence presented, as the liquid sample moved up the paper strips, from the first portion where it was applied to and beyond the second portion. This very motion of the liquid sample is evidence of capillary flow within the permeable material.

That the first portion of the strips shown in the exhibits was the site for application of liquid sample and for "a binder for the analyte coupled to a colored particle" or a "conjugate movably supported therein" is apparent from the Declarations of Dr. Charlton and Ms. Mazzeo. In the reductions to practice of Exhibits A, E, and G, the urine samples containing zero or known hCG concentrations together with the gold conjugate were introduced onto the first portion of the strip by contacting the bottom most portion of the strips with the liquid samples (see page 6, ¶ 21, page 12, ¶ 32, and page 15, ¶ 38 of Ms. Mazzeo's Declaration). In the reductions to practice shown in Exhibit F, the liquid sample was applied the first portion, the discs, with a pipette (see page 18, ¶ 44 of Ms. Mazzeo's Declaration). The gold conjugate is a "binder for the analyte" (antibody to hCG) "coupled to a colored particle" (gold particles) as required by the first two alternate sections of the count. Thereafter, when the mixture began to wick up the strips, the conjugate immediately and necessarily became "movably supported therein" as required by the first two alternate sections of the count as it was contained within the strip and migrated with the liquid sample from the site of application to and beyond the test site. In Exhibit F, the gold conjugate was first dried onto the first portion (discs). As the sample was applied to the first portion and as the liquid sample permeated through the discs to the strips, the gold conjugate was reconstituted and moved to the test site and thus was also "movably supported therein" as required by the first two alternate sections of the count. This is apparent from the plainly visible color development at the test site and loss of color on the discs of the first portion. Accordingly, here also the gold conjugate in the first portion was movably supported.

In all four reductions to practice, shown in Exhibits A, E, F, and G, the second portion was the site for visually determining the presence of the colored particles as required by the first

two alternate sections of the count. The presence of the colored particles, in the second portion, was visually determined, in a positive test, as color developed in the second portion (and indeed can still be visually determined to this day). The second portion, in each reduction to practice, consisted of an antibody (151) adhered to the surface of latex particles which in turn were applied and adhered to the test strip, as recorded in Exhibits D and G. Exhibit G demonstrates that the antibody-coated latex is immobilized onto the strips as required by the first two alternate sections of the count. Exhibit G also shows that the antibody per se, applied to the strip without the latex, does not immobilize sufficiently to enable reproduction of a positive test. Further, in her Declaration at page 4, ¶19, page 10, ¶29, page 14, ¶37 and page 18, ¶ 44, Ms. Mazzeo explains and corroborates that the 151-antibody portion of the antibody-coated latex is a "binder which specifically binds to the analyte," as required by the first two alternate sections of the count, as it is an hCG-specific antibody.

In all four actual reductions to practice shown in Exhibits A, E, F, and G, Ms. Mazzeo declares that before March 27, 1987 she actually performed, in the United States under the direction of inventor Dr. Charlton, a method for determining an analyte, i.e., hCG, in a liquid sample, i.e., urine. Ms. Mazzeo conducted the first step as required in the second section of the count by adding a liquid sample to the first portion of a test device which also satisfy the requirements of the second alternate portion of the count, as demonstrated above, when she contacted the bottom portion of the strip with the liquid sample in each case (see page 6, ¶ 21, page 12, ¶ 32, page 15, ¶ 38, and page 18, ¶ 44 of Ms. Mazzeo's Declaration). Ms. Mazzeo also conducted the second step as required in the second section of the count by waiting a certain amount of time, allowing the liquid to flow from the site of application to the second portion of the permeable material. This occurred in all four experiments by capillary flow and is evidenced by 1) Ms. Mazzeo's observation that all the liquid sample became adsorbed on the strips (see page 6, ¶ 21, page 12, ¶ 32, page 15, ¶ 38, and page 18, ¶ 44 of Ms. Mazzeo's Declaration) and 2) by the development of color at the test sites. Finally, Ms. Mazzeo conducted the last step required by the second alternate section of the count of determining the presence of the analyte, here hCG,

in the liquid sample by effecting a visual inspection of the second portion (the deposit of antibody sensitized latex) for color development (see page 6, ¶ 22, page 13, ¶ 34, page 16, ¶ 40, and page 19, ¶ 45 of Ms. Mazzeo's Declaration). In each case, the presence of analyte was indicated by the presence of colored particles in the second portion of the strips as required by the second section of the count. The colored particles could be seen with the naked eye, and can still be seen to this day as evidenced by the color photostatic copies of the actual devices shown in Exhibits A, E, F, and G, and could be distinguished from hCG-free urine samples.

It should be noted that each and every single device fabricated by Ms. Mazzeo in her experiments shown in Exhibits A, E, F and G, with the exception of the devices coated with only 151 antibody in Exhibit G, is a separate, independent and actual reduction to practice of the subject matter of the first section of the count. Each and every single assay conducted by Ms. Mazzeo in her experiments shown in Exhibits A, E, F and G, with the exception of the devices coated with only 151 antibody in Exhibit G, using a single device, is a separate, independent and actual reduction to practice of the subject matter of the second section of the count. Each Exhibit A, E, F, or G, shows an array of assays conducted by Ms. Mazzeo using various concentrations of analyte, hCG, in the liquid sample. This array of tests is not essential to meet the requirements of the count but illustrate and demonstrate the qualitative and quantitative nature of the devices made and of the method performed.

As a further aid in understanding the experiments, the undersigned attorney attaches copies of the notebook pages identified as Exhibits A, E, F, and G labeled to show the various parts and method steps constituting the elements of the count. As a still further aid, a table setting forth the elements of the count and non-limiting references to the location in the declarations and exhibits of the proofs of the elements and corroboration thereof also is enclosed.

Accordingly, Applicant submits he has successfully shown possession of the invention prior to the Rosenstein Patent filing date and therefore that he is *prima facie* entitled to judgment.

Prompt declaration of an interference is respectfully requested.

Respectfully submitted,

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187ERP1451/1.417906-1

TABLE: DETAILED EXPLANATION

Count: First alternate section	Exhibit A: page 014	Mazzeo Declaration
A test device for determining an analyte in a liquid sample, the device comprising	analyte: hCG	page 4, ¶18, line 11
a permeable material defining at least a first portion and a second portion, the portions being in the same plane as to permit capillary flow communication with each other	a) cellulose acetate (bottom left), and b) glass fiber on gel bond support (bottom right) about1/4 in bottom most portion of strip about 1/2 in, and b) 1 in from bottom most portion of strip a plannar strip	page 4, ¶18, lines 11 and 12; page 5, ¶19; lines 4-8 and 15-19; page 6, ¶21, lines 13-18 Pages 5-6, ¶20
said first portion being the site for application of the liquid sample, and	about1/4 in bottom portion of the strip a) Pregnazyme tris buffer containing hCG (0 and 1 IU/ml) and conjugate (1 OD); b) urine pool containing hCG (0, 50, 150 and 1 IU/ml) and conjugate (1 OD)	page 6, ¶20 page 4, ¶18, lines 11 and 12; pages 4-5, ¶19,; page 6, ¶21
for a conjugate movably supported therein, wherein said conjugate consists of a binder for the analyte coupled to a colored particle, and	Gold15nm-antibody108 conjugate	page 4, ¶19, lines 5-8, and page 5, ¶19, lines 1-4
said second portion being the site for visually determining the presence of the colored particle	a) 1 in from bottom portion of the strip, and b) 1/2 in from bottom portion of the strip;	page 6, ¶22; page 7, ¶24
said second portion consisting of a binder immobilized therein which specifically binds to the analyte	a) and b) : antibody 151-sensitized latex immobilized on the strip.	page 4, ¶18, line 10; ¶19, lines 1-5

TABLE: DETAILED EXPLANATION

Count: First alternate section	Exhibit E: page 25	Mazzeo Declaration
A test device for determining an analyte in a liquid sample, the device comprising	analyte hCG	page 11 ¶32, page 13, ¶34
a permeable material defining at least a first portion and a second portion, the portions being in the same plane as to permit capillary flow communication with each other	glass fiber on gel bond support 1/4 in bottom portion of the strip 1/2 in bottom portion of the strip a plannar strip	page 8, ¶26, line 14; pages 10-11, ¶30-31 page 12, ¶32, lines 14-20 pages 10-11, ¶30-31
said first portion being the site for application of the liquid sample, and	bottom portion of the strip hCG standard solution at 1 IU/ml diluted in negative urine pool (115J056) to 0, 25, 50, 100 mIU and 1 IU	page 12, ¶32, lines 14-20 page 12, ¶32, lines 2-14
for a conjugate movably supported therein, wherein said conjugate consists of a binder for the analyte coupled to a colored particle, and	Gold15nm-antibody108 and Gold30nm-antibody108, both in 1 M K_2PO_4, 1% BSA, 4% PVP, .02 % PEG and 0.1 % NaN_3 pH 7.5	page 12, ¶32, lines 20-22 page 8, ¶27, page 9, ¶27, lines 1-9
said second portion being the site for visually determining the presence of the colored particle	1/2 inch from bottom portion of the strip	page 9, ¶27, lines 18-24; page 10, ¶30, page 12, ¶31 page 12, ¶34
said second portion consisting of a binder immobilized therein which specifically binds to the analyte	antibody 151-sensitized latex immobilized on the strip.	page 9, ¶27, lines 9-24; pages 9-11 ¶28-31

TABLE: DETAILED EXPLANATION

Count: First alternate section	Exhibit F: Page 33	Mazzeo Declaration
A test device for determining an analyte in a liquid sample, the device comprising	analyte: hCG b105 and b108 hCG-antibodies	page 18, ¶44
a permeable material defining at least	disc of Whatman paper with a strip of glass fiber	page 18, ¶44, lines 1-14;
a first portion and	a disc of Whatman paper of either a) GF/D, b) D801B, c) D28, d) 17Chr and e) ? (GF/D) adjoining	page 18, ¶44, lines 1-9
a second portion,	middle portion of the strip of glass fiber	page 18, ¶44, lines 10-11
the portions being in the same plane as to permit capillary flow communication with each other	both disc and strip stuck on gel bond support	page 18, ¶44, lines 9-14
said first portion being the site for application of the liquid sample, and	disk, or cotton ball (cigarette filter) adjoining the disk	page 18, ¶44, lines 14-16
for a conjugate movably supported therein, wherein said conjugate consists of a binder for the analyte coupled to a colored particle, and	a) Gold15nm-antibody105 conjugate and b) Gold30nm-antibody108 b105 and b108 hCG-antibodies	page 17, ¶43, page 18, ¶44, lines 8-9
said second portion being the site for visually determining the presence of the colored particle	middle portion of the strip	page 19, ¶45, lines 1-5
said second portion consisting of a binder immobilized therein which specifically binds to the analyte	antibody 151-sensitized latex immobilized on the strip	page 18, ¶44, lines 10-11

TABLE: DETAILED EXPLANATION

Count: First alternate section	Exhibit G: page 36	Mazzeo Declaration
A test device for determining an analyte in a liquid sample, the device comprising	analyte: hCG	page 16, ¶40
a permeable material defining at least a first portion and a second portion, the portions being in the same plane as to permit capillary flow communication with each other	a plannar strip of Whatman glass fiber paper (GF/A)	page 14, ¶36, line 6; page 15, ¶38 page 14, ¶38, lines 15-18 page 18, ¶44, lines 10-11 page 18, ¶44, lines 9-14
said first portion being the site for application of the liquid sample, and	the liquid sample (250 ul) hCG (Sigma lot#115F-018) in negative urine pool (115J056) 0, 25, 50, and 100 mIU, and 1 and 100 IU premixed with	page 15, ¶38, lines 5-13; page 18, ¶44, lines 14-16
for a conjugate movably supported therein, wherein said conjugate consists of a binder for the analyte coupled to a colored particle, and	conjugate Gold30nm-antibody108 in pH 7.5 1 M K_2PO_4, 1% BSA, 4% PVP, .02 % PEG, 1 % NaN_3, 0.1 M boric acid and 1% Ficoll	page 15, ¶38, lines 9-13; page 15, ¶38, lines 17-20; page 14, ¶37, lines 1-10
said second portion being the site for visually determining the presence of the colored particle	1/2 inch from bottom portion of the strip	page 16, ¶40
said second portion consisting of a binder immobilized therein which specifically binds to the analyte	antibody 151-sensitized latex immobilized on the strip	page 14, ¶36, line 6; page 14, ¶37, lines 9-15; page 15, ¶38, lines 3-5

TABLE: DETAILED EXPLANATION

Count: Second Alternate Section	Exhibit A: page 014	Mazzeo Declaration
A method for determining an analyte in a liquid sample, comprising	analyte: hCG	page 4, ¶18, line 11
a) adding a liquid sample to a first portion of a test device comprising	contacting bottom portion of the strip with the liquid sample	page 6, ¶21, lines 13-15
a permeable material defining at least	a) cellulose acetate (bottom left), and b) glass fiber on gel bond support (bottom right)	page 4, ¶18, lines 11 and 12; page 5, ¶19; lines 4-8 and 15-19;
a first portion and	about1/4 in bottom most portion of strip	page 6, ¶21, lines 13-18
a second portion,	about 1/2 in, and b) 1 in from bottom most portion of strip	Pages 5-6, ¶20
the portions being in the same plane as to permit capillary flow communication with each other	a plannar strip	
said first portion being the site for application of the liquid sample, and for	about1/4 in bottom portion of the strip	page 6, ¶20
	a) Pregnazyme tris buffer containing hCG (0 and 1 IU/ml) and conjugate (1 OD); b) urine pool containing hCG (0, 50, 150 and 1 IU/ml) and conjugate (1 OD)	page 4, ¶18, lines 11 and 12; pages 4-5, ¶19,; page 6, ¶21
a conjugate movably supported therein, wherein said conjugate consists of a binder for the analyte coupled to a colored particle, and	Gold15nm-antibody108 conjugate	page 4, ¶19, lines 5-8, and page 5, ¶19, lines 1-4
said second portion being the site for visually determining the presence of the colored particle	a) 1 in from bottom portion of the strip, and b) 1/2 in from bottom portion of the strip;	page 6, ¶22; page 7, ¶24
said second portion consisting of a binder immobilized therein which specifically binds to the analyte	a) and b) : antibody 151-sensitized latex immobilized on the strip.	page 4, ¶18, line 10; ¶19, lines 1-5
b) allowing the liquid sample to flow to the second portion of the permeable material; and	wait 10 to 30 mins; untill the strips had absorbed the liquid samples	page 6, ¶21, lines 16-18 page 7, ¶22, lines 5-7
c) determining the presence of the analyte in the liquid sample by visual inspection of the second portion for color development, wherein the presence of the analyte is indicated by the presence of colored particles	visual inspection of the test site	pages 7-8, ¶24

TABLE: DETAILED EXPLANATION

Count: Second Alternate Section	Exhibit E: page 25	Mazzeo Declaration
A method for determining an analyte in a liquid sample, comprising	analyte hCG	page 11 ¶32, page 13, ¶34
a) adding a liquid sample to a first portion of a test device comprising	contacting bottom portion of the strip with the liquid sample	page 12, ¶32, lines 14- 20
a permeable material defining at least a first portion and a second portion, the portions being in the same plane as to permit capillary flow communication with each other	glass fiber on gel bond support 1/4 in bottom portion of the strip 1/2 in bottom portion of the strip a plannar strip	page 8, ¶26, line 14; pages 10-11, ¶30-31 page 12, ¶32, lines 14-20 pages 10-11, ¶30-31
said first portion being the site for application of the liquid sample, and for	bottom portion of the strip hCG standard solution at 1 IU/ml diluted in negative urine pool (115J056) to 0, 25, 50, 100 mIU and 1 IU	page 12, ¶32, lines 14-20 page 12, ¶32, lines 2-14
a conjugate movably supported therein, wherein said conjugate consists of a binder for the analyte coupled to a colored particle, and	Gold15nm-antibody108 and Gold30nm-antibody108, both in 1 M K_2PO_4, 1% BSA, 4% PVP, .02 % PEG and 0.1 % NaN_3 pH 7.5	page 12, ¶32, lines 20-22 page 8, ¶27, page 9, ¶27, lines 1-9
said second portion being the site for visually determining the presence of the colored particle	1/2 inch from bottom portion of the strip	page 9, ¶27, lines 18-24; page 10, ¶30, page 12, ¶31 page 12, ¶34
said second portion consisting of a binder immobilized therein which specifically binds to the analyte	antibody 151-sensitized latex immobilized on the strip.	page 9, ¶27, lines 9-24; pages 9-11 ¶28-31
b) allowing the liquid sample to flow to the second portion of the permeable material; and	wait 10 to 30 mins; untill the strips had absorbed the liquid samples	page 12, ¶32, lines 20-23
c) determining the presence of the analyte in the liquid sample by visual inspection of the second portion for color development, wherein the presence of the analyte is indicated by the presence of colored particles	visual inspection of the test site	page 13, ¶34

TABLE: DETAILED EXPLANATION

Count: Second Alternate Section	Exhibit F: Page 33	Mazzeo Declaration
A method for determining an analyte in a liquid sample, comprising	analyte: hCG b105 and b108 hCG-antibodies	page 18, ¶44
a) adding a liquid sample to a first portion of a test device comprising	droppind liquid sample onto the discs with a pipette	page 18, ¶44, lines 14-16
a permeable material defining at least a first portion and a second portion, the portions being in the same plane as to permit capillary flow communication with each other	disc of Whatman paper with a strip of glass fiber a disc of Whatman paper of either a) GF/D, b) D801B, c) D28, d) 17Chr and e) ? (GF/D) adjoining middle portion of the strip of glass fiber both disc and strip stuck on gel bond support	page 18, ¶44, lines 1-14; page 18, ¶44, lines 1-9 page 18, ¶44, lines 10-11 page 18, ¶44, lines 9-14
said first portion being the site for application of the liquid sample, and for	disk, or cotton ball (cigarette filter) adjoining the disk	page 18, ¶44, lines 14-16
a conjugate movably supported therein, wherein said conjugate consists of a binder for the analyte coupled to a colored particle, and	a) Gold15nm-antibody105 conjugate and b) Gold30nm-antibody108 b105 and b108 hCG-antibodies	page 17, ¶43, page 18, ¶44, lines 8-9
said second portion being the site for visually determining the presence of the colored particle	middle portion of the strip	page 19, ¶45, lines 1-5
said second portion consisting of a binder immobilized therein which specifically binds to the analyte	antibody 151-sensitized latex immobilized on the strip	page 18, ¶44, lines 10-11
b) allowing the liquid sample to flow to the second portion of the permeable material; and	wait 10 to 30 mins; until the strips had absorbed the liquid samples	page 19, ¶45
c) determining the presence of the analyte in the liquid sample by visual inspection of the second portion for color development, wherein the presence of the analyte is indicated by the presence of colored particles	visual inspection of the test site	page 19, ¶45

TABLE: DETAILED EXPLANATION

Count: Second Alternate Section	Exhibit G: page 36	Mazzeo Declaration
A method for determining an analyte in a liquid sample, comprising	analyte: hCG	page 16, ¶40
a) adding a liquid sample to a first portion of a test device comprising	contacting bottom portion of the strip with the liquid sample	
a permeable material defining at least a first portion and a second portion, the portions being in the same plane as to permit capillary flow communication with each other	a plannar strip of Whatman glass fiber paper (GF/A)	page 14, ¶36, line 6; page 15, ¶38 page 14, ¶38, lines 15-18 page 18, ¶44, lines 10-11 page 18, ¶44, lines 9-14
said first portion being the site for application of the liquid sample, and for	the liquid sample (250 ul) hCG (Sigma lot#115F-018) in negative urine pool (115J056) 0, 25, 50, and 100 mIU, and 1 and 100 IU premixed with	page 15, ¶38, lines 5-13; page 18, ¶44, lines 14-16
a conjugate movably supported therein, wherein said conjugate consists of a binder for the analyte coupled to a colored particle, and	conjugate Gold30nm-antibody108 in pH 7.5 1 M K_2PO_4, 1% BSA, 4% PVP, .02 % PEG, 1 % NaN_3, 0.1 M boric acid and 1% Ficoll	page 15, ¶38, lines 9-13; page 15, ¶38, lines 17-20; page 14, ¶37, lines 1-10
said second portion being the site for visually determining the presence of the colored particle	1/2 inch from bottom portion of the strip	page 16, ¶40
said second portion consisting of a binder immobilized therein which specifically binds to the analyte	antibody 151-sensitized latex immobilized on the strip	page 14, ¶36, line 6; page 14, ¶37, lines 9-15; page 15, ¶38, lines 3-5
b) allowing the liquid sample to flow to the second portion of the permeable material; and	wait 10 to 30 mins; untill the strips had absorbed the liquid samples	page 15, ¶38, lines 13-17
c) determining the presence of the analyte in the liquid sample by visual inspection of the second portion for color development, wherein the presence of the analyte is indicated by the presence of colored particles	visual inspection of the test site	page 15, ¶38, lines 17-20

PATENT
Atty. Docket No. CWP-012CN
(1451/2)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): Charlton et al.

SERIAL NUMBER: 08/465,675 GROUP NUMBER: 1817

FILING DATE: June 6, 1995 EXAMINER: C. A. Spiegel

TITLE: Test Device and Method For Colored Particle Immunoassay

DECLARATION OF DAVID E. CHARLTON

I, DAVID E. CHARLTON, Ph.D., declare:

1. I am currently the President of Bionike Incorporated located at 1015 Grandview Drive, South San Francisco, California 94080-4910.

2. I received a Bachelor's Degree in biology and microbiology from the University of Windsor, in Windsor, Ontario in 1972. In 1973, I received my Masters of Science from the University of Windsor, specializing in virology. In 1977, I attained a Ph.D. from the University of Toronto in immunology.

3. I began working at Carter-Wallace, Inc. ("Carter-Wallace") in the Carter Products Division, New Jersey, USA, in 1985 as Manager of Product Development. In that position, my job responsibilities included developing diagnostic tests for the "over the counter" ("OTC") market.

4. During the course of my employment at Carter-Wallace, I worked on the development of a quick, easy to perform diagnostic test for the detection of Human Chorionic Gonadotropin (hCG) in urine. This project was referred to within Carter-Wallace as the "Easy Assay" project.

5. I make the declaration herein based on my recollection of work done at Carter-Wallace under my direction at a time prior to March 27, 1987, as refreshed by review of various documents attached hereto.

6. Prior to March 27, 1987, in the United States of America, I conceived of a device for determining the presence of an analyte in a liquid sample. This device was to be made of a strip of permeable material capable of transporting by wicking or capillary action a liquid sample, such as urine, suspected to contain the analyte of interest, such as human Chorionic Gonadotropin (hCG) or luteinizing hormone (LH). The strip was to include a first portion which would be the site of application of the liquid sample and for a conjugate composed of a colored particle coupled to a binder, e.g., an antibody, for the analyte, and a second portion having another binder for the analyte immobilized on the permeable material. The immobilized binder for the analyte was to permit the determination of the presence of the colored particle in the second portion by formation, in the presence of an analyte, of a sandwich complex. This invention became part of a larger project at Carter-Wallace called "Easy Assay."

7. Initially, the Easy Assay project contemplated several different pregnancy test formats. In particular, products being developed within the Easy Assay project embodied: (1) "platform"; (2) "dipstick" (also called "chromatostrip" or "chromatostick"); and (3) "pee-on" formats. Of those three, the "dipstick" and the "pee-on" correspond to the invention claimed in patent application serial No. 08/465,675 filed June 6, 1995 of which I am a named co-inventor. These latter two formats differed only in the location of the conjugate used in the test. In the "dipstick," the conjugate was present in dried form in a cup or vial, and was reconstituted when urine was added. In the "pee-on," the conjugate was present in dried form on a portion of the strip upstream of the test site, and was reconstituted when urine passed along the strip.

8. In particular, both the Easy Assay "dipstick" and "pee-on" formats are chromatographic tests using colloidal gold labeled antibody, and a capturing antibody immobilized within a zone of a membrane. In both these formats, I envisioned that the test would "conduct itself," by enabling urine to wick through and along the membrane to the immobilized antibody, where, in the presence of an analyte, the gold would collect to provide a colored signal. I was familiar with the use of colloidal gold as a signaling agent from prior work I had been involved in. Also, methods for preparing colloidal gold conjugates were described in US patent serial no. 4,313,734 to Leuvering, a copy of which is attached as **Exhibit M**, as disclosed in the specification of the instant patent application at page 15, second paragraph. I discussed my ideas with Neil Miller, another Carter-Wallace employee, and together we developed test kit formats for carrying out "dipstick" and "pee-on" assays.

9. Ms. Margaret Verbanic (now Mazzeo), a research technician with the Carter International Group, joined the Easy Assay project when she started working for me. Ms. Mazzeo's responsibilities with regard to the Easy Assay project included carrying out experiments at my direction. Her experiments were directed to identifying the feasibility of test formats, and to optimizing the reagents and other parameters of the tests.

10. While working on the Easy Assay project, I met with Ms. Mazzeo at the Carter-Wallace laboratory almost every morning, to discuss the experiments Ms. Mazzeo had performed, and to identify future areas of research. Ms. Mazzeo reported to me the results of the experiments she had conducted, and we discussed them.

11. Prior to March 27, 1987, Ms. Mazzeo carried out "dipstick" and "pee-on" format pregnancy tests at my direction. For example Ms. Mazzeo's laboratory notebook shows that she

carried out a series of assays which included all of the elements of the "dipstick" invention at several occasions.

12. For example, Page No. 1608-014 ("page 014") of Ms. Mazzeo's notebook, a copy of which is attached hereto as **Exhibit A**, shows that prior to March 27, 1987 Ms. Mazzeo reduced to practice the "dipstick" concept I described to her. To conduct the experiment recorded at page 014, Ms. Mazzeo fabricated "dipsticks" made of strips of glass-fiber paper bound to gel bond supports with sensitized latex particles immobilized on a portion of the strips. The latex particles were "sensitized" with anti-hCG antibody called B-151. The use of latex particles coated with antibody was commonly known at the time as an effective technique for immobilizing antibody on a support. This technique also had the advantage of increasing the surface area available to support the antibody within the same volume, thus providing greater sensitivity for the assay. The latex particles were immobilized on the paper by applying a liquid formulation of anti-hCG antibody sensitized latex to the paper, and allowing the formulation to dry on the paper.

13. As noted on **Exhibit A**, next, Ms. Mazzeo used a solution of colloidal gold conjugate which she diluted to a concentration of 1 "optical density" unit per milliliter as determined by spectrophotometry at 525 nanometer (nm) wavelength using urine samples containing known quantities of hCG. The colloidal gold conjugate included colloidal gold particles of 15 nm average size labeled with hCG-specific antibody, called B-108.

14. Next in her experiment, Ms. Mazzeo introduced a small portion of the bottom of each dipstick into a urine sample containing a known amount of hCG and gold conjugate. The bottom portions of the dipsticks contacted with the liquid samples were of a length sufficient to allow the liquid samples to wick up the strips toward the portions of the dipsticks having the immobilized antibody-coated latex. Ten minutes later, as noted

in her notebook page 014 shown in **Exhibit A**, Ms. Mazzeo observed the development of a colored spot on the glass-fiber papers which is indicative of the presence of hCG. Ms. Mazzeo removed the dipsticks from the sample after 30 minutes and placed them in her notebook for her record.

15. Ms. Mazzeo's experiment described in **Exhibit A** demonstrated that the "dipstick" test format worked. The dipsticks made by Ms. Mazzeo and used in this experiment constituted an actual reduction to practice of the device I conceived and which is claimed in pending patent application serial No. 08/465,675. Ms. Mazzeo's use of the devices in carrying out the experiment constituted an actual reduction to practice of the method I conceived which is claimed in pending patent application serial No. 08/465,675. A difference in color intensity between any two strips is detectable by direct observation of the dipsticks at the test sites. The results of this experiment clearly indicate an assay that is specific to the presence of hCG. This experiment demonstrated a qualitative and quantitative means for determining the presence of hCG in the liquid samples. The qualitative determination is made by direct observation of the color intensity developed at the test sites between the dipstick used in the control liquid sample, with no hCG present, and the dipstick used in a liquid sample containing a known amount of hCG, both against the color background of the strips, pale pink. The quantitative determination is made by direct observation of the color intensity gradient, from pale to dark pink, developed on the dipsticks at the test sites in proportion to the increase in the concentration of hCG present in the liquid samples. The assays conducted by Ms. Mazzeo and recorded at page 014, attached as **Exhibit A**, show that the sensitivity of this assay format permitted the determination of the presence of hCG at a concentration as low as 50 mIU/ml.

16. I presented the results of this experiment, described in **Exhibit A**, and some other subsequent experiments carried out by Ms. Mazzeo, at a "Gold sol Membrane Meeting" held at Carter-Wallace prior to March 27, 1987. The "Gold sol Membrane Meetings" were held regularly at Carter-Wallace to monitor the development and plan production of the "Easy Assay" project. The content of this meeting was summarized and recorded in a memorandum to "Distribution" from Melinda Goddard, also an employee of Carter-Wallace who attended the meeting, a true and correct copy of which is attached hereto as **Exhibit H** except that all the dates appearing thereon have been redacted. I have reviewed an unredacted copy of this exhibit and it bears dates earlier than March 27, 1987.

17. As can been seen in **Exhibit H**, my name appears in the list of attendees and in the distribution list. I attended the meeting referred to in the memo and received a copy of the memo on or shortly after the time of its date. To the best of my recollection, I believe that it accurately records an outline of what was discussed at the meeting. Under the heading "Colloidal Gold Concepts," subheading 2., the memo relates an accurate summary of the progress of our work on the dipstick assay format. In particular, it states that "lyophilized conjugate which is reconstituted with the urine sample (in a tube) into which a wicking strip is placed then read after 5 minutes for color development with as little as 25 mIU/ ml of hCG." the "lyophilized conjugate" refers to a gold particle coated with hCG-specific antibody. The "wicking strip" refers to a dipstick made of a piece of permeable glass fiber paper bound to gel bond support and with hCG-specific antibody-coated latex. "Color development" refers to the accumulation of colored gold particles at the test site, where the hCG-specific antibody-coated latex is immobilized, in the form of a sandwich complex (gold-antibody/hCG/antibody-latex).

18. After reducing the dipstick technology to practice,

Ms. Mazzeo and I worked toward enhancing the speed, accuracy, and sensitivity of this technology. Toward this goal and still prior to March 27, 1987, Ms. Mazzeo and I conducted additional experiments for the evaluation of the Easy Assay "dipstick" technology. Ms. Mazzeo and I worked on optimizing the various components of the test system, such as size of the gold particle and the antibody system, and increasing the sensitivity of the antibody on the latex. This is reflected in Ms. Mazzeo's laboratory notebooks and other documents.

19. For example, page 1608-025 ("page 025") of Ms. Mazzeo's notebook, a copy of which is attached hereto as **Exhibit E**, shows that, prior to March 27, 1987, Ms. Mazzeo again reduced to practice the "dipstick" concept I had described to her. This experiment was designed to assess the changes in sensitivity of the assay in function of the size of the gold particle. For this, Ms. Mazzeo prepared two gold-antibody conjugates: one with 30 nanometer (nm) size for the gold particles, noted as "b108 $gold_{30nm}$" in **Exhibit E**, the other with 15 nm size, noted as "b108 $gold_{15}$." Then, Ms. Mazzeo prepared the dipsticks by applying a formulation of hCG-specific antibody coated latex onto the glass-fiber paper bound to gel bond support following an optimized procedure she had earlier developed (as indicated on **Exhibit E** by the reference to page 024 of her notebook).

20. Next, Ms. Mazzeo prepared urine samples containing known amounts of hCG by diluting a standard solution of hCG with a negative urine pool. She then used 500 microliters (µl) of each urine samples thus prepared to reconstitute the gold conjugates, and transferred 250 µl of each liquid sample into a clean tube before contacting one end of the dipsticks with the liquid samples. Ms. Mazzeo maintained the dipsticks in contact with the liquid samples until all the fluid was absorbed. She then placed the dipsticks in her notebook at page 025 for her record.

21. The experiment recorded at page 025 and shown in **Exhibit E** again evidences a reduction to practice of the "dipstick" concept I had described to Ms. Mazzeo. In particular, the dipsticks made by Ms. Mazzeo and used in this experiment constituted an actual reduction to practice of the device I conceived and which is claimed in pending patent application serial No. 08/465,675. Ms. Mazzeo's use of the devices in carrying out the experiment constituted an actual reduction to practice of the method I conceived which is claimed in pending patent application serial No. 08/465,675. The dipsticks having the 30-nm-size gold conjugate, b108-$gold_{30}$, developed deeper shades of pink color (the five strips shown on the bottom left corner of page 025, shown as **Exhibit E**) than did the dipsticks having the 15-nm-size gold conjugate, b108-$gold_{15}$ (the five strips shown on the bottom right corner of page 025, shown as **Exhibit E**). Nonetheless, both sets of strips also clearly indicate an assay that is specific to the presence of hCG. An increase in color intensity between any two strips, within each set of strips, is detectable by direct observation of the dipsticks at the test sites. Again, this experiment shows that the dipstick assay format provided both a qualitative and a quantitative indication of the presence of hCG in a liquid sample. The qualitative determination is made by direct observation of development of color at the test sites with the dipstick contacted with a liquid sample containing a known amount of hCG, viewed against the white color background of the strips and no color development at the test site of the dipstick contacted with the control liquid sample, with no hCG present. A quantitative determination was made by direct observation of the color intensity gradient, from pale to dark pink, developed within each set of dipsticks at the test sites in proportion to the increase in the concentration of hCG present in the sets of liquid samples. The presence of hCG was detected with as little as 25 mIU/ml of hCG in the urine samples with both sizes of gold conjugate.

22. After this experiment, Ms. Mazzeo and I continued working on the "dipstick" assay format to determine all the parameters needed of an assay product for commercialization in the "over-the-counter" (OTC) market. In that regard, we investigated the sensitivity limits of the "dipstick" assay format, still prior to March 27, 1987. The maximum capacity is the highest analyte concentration that can be detected using a set of conditions (e.g., the amount of binding sites for hCG available on gold conjugate and antibody-coated latex reagents) for the "dipstick" assay format and beyond which the system no longer operates to visualize the presence of the analyte. Optimum conditions are reached when there is an excess of capturing sites immobilized on the strips over the amount of analyte in the liquid sample. This assures that at least some of the analyte will be bound to both antibodies, in the form of a full sandwich. The formation of the full sandwich (gold-antibody/hCG/antibody-latex) at the test site permits the detection of the presence of the analyte by direct observation of the color development due to the immobilization of the gold particle that occurs in the formation of the sandwich complex. Beyond the optimum conditions, when there exists an excess of the analyte over both the amount of gold conjugate and the amount of capturing sites immobilized on the strips, the analyte starts competing with the complex (gold conjugate/analyte) for the capturing sites and thus inhibits the formation of the sandwich complex at the test site. This phenomenon represents an overloading of the system and is called "high dose hook" because, beyond the optimum point, the color intensity that develops at the test site tends to decrease with the increase of the concentration of the analyte. During the development of the "dipstick" assay format, and still prior to March 27, 1987, I directed Ms. Mazzeo to carry certain experiments to determine the optimum conditions for the detection of hCG. This is reflected in Ms. Mazzeo's notebook.

23. For example, page 1608-036 ("page 036") of Ms.

Mazzeo's notebook, a copy of which is attached hereto as **Exhibit G**, shows that Ms. Mazzeo once again reduced to practice the "dipstick" concept prior to March 27, 1987. Ms. Mazzeo and I designed the experiment described in page 036 and shown in **Exhibit G** to assess the maximum capacity of the "dipstick" assay system. Once again Ms. Mazzeo prepared dipsticks made of strips of glass-fiber paper bound to gel bond support with hCG-specific antibody sensitized latex immobilized on the strips. Ms. Mazzeo used "b108 $gold_{30nm}$" as gold conjugate in lyophilized form. Ms. Mazzeo prepared urine samples containing known amount of hCG; here the concentration of hCG in the samples ranged from 25 mIU/ml to 100 IU/ml.

24. As noted in **Exhibit G**, to conduct this experiment, Ms. Mazzeo used an amount of urine sample containing a known amount of hCG to reconstitute the gold conjugate, transferred 250 µl of this mixture to a clean tube, and introduced the bottom portion of a dipstick below the test site into the liquid sample. Ms. Mazzeo repeated this for the five concentrations of hCG and the control. Ms. Mazzeo waited ten minutes before reading the results. Her observations are recorded on page 036 shown in **Exhibit G**. The actual dipsticks used in this test are secured to the bottom right corner of that page.

25. The experiment described at page 036, shown in **Exhibit G**, again constituted a reduction to practice of the "dipstick" test format. In particular, the dipsticks made by Ms. Mazzeo and used in this experiment constituted an actual reduction to practice of the device I conceived and which is claimed in pending patent application serial No. 08/465,675. Ms. Mazzeo's use of the devices in carrying out the experiment constituted an actual reduction to practice of the method I conceived which is also claimed in pending patent application serial No. 08/465,675. An increase in color intensity was and still is detectable by direct observation of the color developed at the dipsticks' test sites, for the dipsticks used in the

urine samples containing a concentration of hCG between 25 mIU/ml to 1 IU/ml. However, no color developed on the dipstick used on the control liquid sample, containing no hCG, or on the dipstick used on the liquid sample containing 100 IU/ml of hCG (due to the high dose hook phenomenon). Once again, the results of this experiment clearly indicate an assay that is specific to the presence of hCG up to an hCG concentration of 1 IU/ml. This experiment also provided a qualitative and quantitative means of determining the presence of hCG in the liquid samples. Similarly to the other experiments, a qualitative determination was made by direct observation of the color intensity developed at the test sites. A quantitative determination was made by direct observation of the color intensity gradient, from pale to dark pink, developed on the dipsticks at the test sites as a result of the increase in the concentration of hCG present in the liquid samples from 0 to 1 IU/ml. The presence of hCG was detected with as little as 25 mIU/ml of hCG in the urine samples, but could no longer be detected at 100 IU/ml.

26. Following the experiments described in **Exhibits E** and **G**, and still prior to March 27, 1987, I again presented the results of the work carried out in my laboratory up to that time at one of our "Gold Sol Membrane Meetings." The content of this meeting was summarized and recorded in a memorandum to "Distribution" from Ms. M. Goddard, an attendee at the meeting. A true and correct copy of this memo is attached hereto as **Exhibit J**, except that all the dates appearing thereon have been redacted. I have reviewed an unredacted copy of this exhibit and it is dated earlier than March 27, 1987.

27. As can be seen on **Exhibit J**, my name appears in the list of attendees and in the distribution list. I attended the meeting referred to in this memo and received a copy of this memo on or shortly after the time of its date. I believe that it accurately records an outline of what was discussed at the meeting. On page one of **Exhibit J**, under the heading

"'Chromatograph' Direct Application Concept Status," subheading 1., the memo describes accurately a summary of the procedure developed in my laboratory and tested by Ms. Mazzeo which I described at the subject Gold Sol Membrane meeting. In particular, it states that "using b108 and 30nm gold, samples of the test was shown to function as follows: a. lyophilize gold in tube; b. add urine; c. place membrane/stick into tube; and d. wait 2-5 minutes and read." The "b108" and "30nm gold" refers to the gold-antibody conjugate specific to hCG. the "urine" refers to a liquid sample containing an amount of hCG. The "membrane/stick" refers to the dipstick fabricated from glass-fiber paper on gel bond with hCG-specific antibody coated latex. The "wait and read" refers to the time required before a direct observation of the color development at the test site can be made. On page two of **Exhibit J**, under subheading 2., the memo further relates some specific issues pertaining to the production of the "dipstick" devices which I described at the meeting. Specifically, it states that "The concept would incorporate die-cut plastic instead of molds and would expose reading 'bars' which would be pre-coated with latex using a jet-spray process." The "latex" here refers to the formulation of hCG-specific antibody, b151, coated on latex particles.

28. Further, still prior to March 27, 1987, I prepared for Carter-Wallace's marketing department a brief summary of the "dipstick" test format in a comparison analysis with a product then sold by a company called New Horizons, for the purpose of demonstrating the superiority of the "Easy Assay" dipstick technology over the New Horizon technology. A true and correct copy of this document is attached hereto as **Exhibit K** except that the date, which precedes March 27, 1987, has been redacted.

29. The first page of **Exhibit K**, includes a table I prepared comparing the New Horizon and the Easy Assay devices. The table has three columns: the first column on the left lists the parameters for both devices; the second column describes the

corresponding elements of the New Horizon device; and the third column lists the corresponding elements of the Easy Assay device we had developed prior to March 27, 1987. For the Easy Assay device, in the row for the solid phase, I listed that the device comprises glass-fiber paper with an antibody attached to latex dried onto it. In the row for the device configuration, I drew two views of the device, a frontal and a lateral, depicting the portion of the strip where the latex coated with the antibody is immobilized on the glass fiber. In the row for the conjugate, I listed the 15 nm gold particle with B108 or B105 antibody, and indicated that the conjugate is provided in a lyophilized form in a test tube. In the row for the procedure, I described the steps to be taken to conduct the test: 1) "reconstitute the gold conjugate with urine;" and 2) "place the dipstick into the gold conjugate-urine mixture and let [the dipstick] absorb [the liquid sample] for a maximum time of 15 minutes." In the last two rows, I recorded the numbers of steps necessary to carry out the test, namely, two steps for the Easy Assay, and the time required to carry out the test, 1 to 15 minutes.

30. Concurrently with the development of the dipstick assay and also prior to March 27, 1987, I conceived of and designed a test having a "pee-on" format. Following my description of the concept to him, Neil Miller prepared a drawing of a prototype for the "pee-on" device including his design for a functional casing. Afterward, Neil Miller and I presented the pee-on device with the drawing of the prototype at a Gold Sol Membrane Meeting prior to March 27, 1987. The content of this meeting was summarized also in a memorandum and circulated to "Distribution" from Ms. M. Goddard, an attendee at this meeting, prior to March 27, 1987, a true and correct copy of which is attached hereto as **Exhibit I** except that all the dates appearing thereon have been redacted. I have reviewed the unredacted copy, and declare that it bears a date that precedes March 27, 1987.

31. As can be seen in **Exhibit I**, my name and the name of Neil Miller appear on the list of attendees and on the memo distribution list. I attended the meeting referred to in this memo and received a copy of this memo on or shortly after the time of its date. I believe that it accurately records an outline of what was discussed at the meeting. On page two of **Exhibit I**, under the heading "Concept Presentation: 'Easy Assay' Direct Sample Application Test," to the best of my recollection, the memo relates an accurate summary of the content of the discussion held at the meeting concerning the presentation of the "pee-on" concept. In particular, under subheading 2., the memo describes that "the device wicks the sample through pre-filled conjugate zone and both control and patient reading zones." The word "sample" refers to a urine sample suspected to contain an analyte such as hCG or LH. The word "conjugate" refers to a gold analyte-specific-antibody conjugate. The words "patient reading zone" refers to the test site having an analyte-specific antibody immobilized in the solid support which is visible to the user.

32. Page three of **Exhibit I** shows the drawings of the "pee-on" concept made by Neil Miller. These drawings show two figures: one depicting the outer casing of the device with an absorbent portion exposed to the outside; and the other depicting the strip contained within the device having a portion for the immobilization of the reagents, such as the antibody-coated latex, and another portion for the conjugate, analyte-specific antibody conjugated to a gold particle.

33. Following the presentation of this concept at the Gold Sol Membrane Meeting, and prior to March 27, 1987, with the assistance of Ms. Mazzeo, I developed a device for the "pee-on" assay format. For example, Page 1608-033 ("page 033") of Ms. Mazzeo's notebook, a copy of which is attached hereto as **Exhibit F**, with all the dates appearing thereon redacted, shows that,

prior to March 27, 1987, Ms. Mazzeo reduced to practice the "pee-on" assay format I had described to her.

34. To conduct the experiment described and recorded at page 033 and shown as **Exhibit F**, Ms. Mazzeo prepared two series of disks cut out of various Whatman™ papers. Then, Ms. Mazzeo dried a gold conjugate on each pair of disks, namely either $gold_{15}$-105 or $gold_{30}$-108. Next, Ms. Mazzeo mounted one of each pair of disks on gel bond support adjoining 1) a strip of glass-fiber paper containing 151-sensitized latex embedded at the test site, such that at least a portion of the disk would be coplanar to the strip for each device, and 2) another absorbent material made of either cigarette filter or blotter paper. Ms. Mazzeo then contacted the absorbent portion of each devices with a liquid sample and removed the devices from the liquid sample after all the liquid had absorbed. As shown on **Exhibit F**, Ms. Mazzeo made a record of this experiment by securing the actual devices matched with their respective spare disks on page 033, and noted beneath each spare disk the type of paper used.

35. The experiment described at page 033, shown in **Exhibit F**, demonstrated that the "pee-on" test format worked. In particular, the dipsticks made by Ms. Mazzeo and used in this experiment constituted another actual reduction to practice of the device I conceived and which is claimed in pending patent application serial No. 08/465,675. Ms. Mazzeo's use of the devices in carrying out the experiment also constituted an actual reduction to practice of the method I conceived which is claimed in pending patent application serial No. 08/465,675. In particular, it demonstrated that the liquid sample moving along the test strip could pick up gold conjugate that had been dried on the support, as it did when the conjugate was premixed with the liquid sample in a container, and entrain it to the test site where the conjugate would bind. This can be seen by 1) the loss of color on the disks portion of the devices compared to

the spare disks, and 2) the color developed at the test sites, on the strip portion of the devices.

36. Following the results of the experiment shown in **Exhibit F**, and still prior to March 27, 1987, I prepared a report for Carter-Wallace's marketing department with a summary of the product line in development in my laboratory with regard to the potential licensing of certain monoclonal antibody technology. A true and correct copy of this report is shown in **Exhibit L**, except that the date has been redacted. I have reviewed an unredacted copy of this memo and it bears a date that is prior to March 27, 1987.

37. As can be seen on **Exhibit L**, I prepared a table that lists the components of several devices that were already in production phase but for which new developments were still on-going, and devices still in development. Among the devices listed are two versions of the "Easy Assay" formats one for hCG and the other for LH. For both, I mentioned 1) "< 15 minutes" which indicates a test time of less than fifteen minutes, 2) "1 step" which indicates a device exploiting the "pee-on" format, 3) "monoclonal Ab. trap" which refers to the b-151 antibody coated on the latex used as capturing agent in the device, and 4) "monoclonal Ab. conj." which refers to either b105 or b108 antibody conjugated to the gold particle used as the marker in the device.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: Oct 9, 1997 David E. Charlton

David E. Charlton

PATENT
Atty. Docket No. CWP-012CN
(1451/2)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): Charlton et al.

SERIAL NUMBER: 08/465,675 GROUP NUMBER: 1817

FILING DATE: June 6, 1995 EXAMINER: C. A. Spiegel

TITLE: Test Device and Method For Colored Particle Immunoassay

DECLARATION OF MARGARET M. MAZZEO

I, MARGARET M. MAZZEO (nee Verbanic), declare:

1. I currently reside at 3 Lovell Drive, Plainsboro, New Jersey 08536. I am presently employed by Novo Nordisk Pharmaceuticals Inc., Princeton, New Jersey as a Statistician.

2. I received my Bachelors of Science Degree in Microbiology and Medical Technology from California Polytechnic University, Pomona, California. In 1993, I received a Masters of Science Degree in Statistics from Rutgers University, New Brunswick, New Jersey.

3. Prior to joining Novo Nordisk Pharmaceuticals Inc., I was employed for 3 1/4 years at Hoechst Marion Roussel Pharmaceuticals, Somerville, New Jersey, as a biostatistician. Prior to joining Hoechst, I was employed for approximately 8 3/4 years by Carter-Wallace, Inc. ("Carter-Wallace").

4. I began working at Carter-Wallace in August, 1984 as an Assistant Scientist in the company's International Division's Research and Development. My responsibilities included conducting experiments in the development of immunoassays for

the detection in serum and urine of antigens including human Chorionic Gonadotropin (hCG) and Luteinizing Hormone (LH). In 1986, I was promoted to Associate Research Scientist and my responsibilities remained essentially the same.

5. In August of 1988, I was promoted to the position of Clinical Studies Coordinator for Diagnostics Research and Development. In that position, my responsibilities included the design, development and management of clinical trials for diagnostics products. I remained in this position for 4 1/2 years.

6. As a scientist, I worked for a time under the direction of Dr. David Charlton, Manager of Product Development, Carter Products Division.

7. Under Dr. Charlton's direction, I worked on Carter-Wallace's "Easy Assay" Project. Easy Assay was the Carter Products Division's general name for an easy-to-perform diagnostic test for the detection of hCG or LH in urine. A goal of the project was to develop a test having minimal steps and requiring little, if any, timing.

8. Initially, the Easy Assay project contemplated three test formats, namely (1) the "platform," (2) the "dipstick," and (3) the "pee-on" formats.

9. The "platform" format was a flow-through membrane assay resembling a disc.

10. The "dipstick" format was also sometimes referred to as the "chromatostick" or "chromatostrip," and utilized a chromatographic test strip held in an elongate plastic housing. The test included a lyophilized reagent including colloidal gold-labeled anti-hCG antibody. In use, the reagent was reconstituted with test urine in a tube, and the bottom of test strip was contacted the urine.

11. The "pee-on" format was intended to be the next generation of the "dipstick" test. This format resembled the "dipstick" test device, but included colloidal gold reagent lyophilized onto a portion of the test strip and in the flow path of the liquid sample. This format contemplated a liquid sample, urine, being applied directly to the test device without first mixing the sample with the colloidal gold reagent.

12. I worked on research and development of the Easy Assay "dipstick" test from the initial concept development through product launch. During the initial phases of research and development, Dr. Charlton, Neil Miller and I worked on this product.

13. I carried out my responsibilities on the Easy Assay project under Dr. Charlton's direction. Dr. Charlton's management style was fairly informal. He would present suggested experiments, general protocols, and instructions, and I would then implement and conduct the experiments. We discussed the results of such experiments and new ones on a regular basis, essentially every working day. In many cases Dr. Charlton and I developed experimental protocols together, and carried out the experiments together.

14. I documented my work on the Easy Assay "dipstick" project in part in my laboratory notebooks and occasionally summarized that work in memos. I also kept Dr. Charlton apprised of the status of my work on this project at all times.

15. Prior to March 27, 1987, Dr. Charlton described to me the Easy Assay "dipstick" test concept. He explained to me that he envisioned a chromatographic-type test, in which a consumer would simply introduce a test device to a sample and let the test "conduct itself."

16. The initial experiments we conducted to demonstrate the feasibility of the "dipstick" concept used materials and reagents that were available in our research laboratory

(reagents, filter papers), several of which were manufactured by Carter-Wallace in connection with production of existing test kits.

17. Prior to March 27, 1987, under Dr. Charlton's direction, I practiced the Easy Assay "dipstick" test on a number of occasions. For example, attached **Exhibits A-G** are true and correct copies of certain pages of laboratory notebook #1608 I maintained during my work under Dr. Charlton's direction except that all dates appearing thereon have been redacted. Everyday, I would record the experiment I was conducting that day in my current notebook essentially contemporaneously with my executing the experiment or shortly thereafter.

18. In particular, **Exhibit A** is a true and correct copy of page 1608-014 ("page 014") of my laboratory notebook #1608, except that the date has been redacted. I have reviewed an unredacted copy of this exhibit and it bears dates that precede March 27, 1987. I handwrote this page, entitled "Test Feasability of a Chromatograph Gold Assay," essentially contemporaneously with my executing the Easy Assay "dipstick" test format described therein and prior to March 27, 1987, to make a record of the experiment. I carried out the experiment in the United States of America using "151 sensitized latex," "$gold_{15}$ 108," "hCG standards," "Cellulose acetate," "pregnazyme tris buffer," "glass fiber paper," and "negative urine pool." I listed these materials on the top half of the page 014, attached as **Exhibit A**, under the heading "Materials."

19. The "151 sensitized latex" notation refers to a formulation of latex particles coated with anti-hCG antibody, specifically, B-151 antibody that was used in products manufactured and marketed by Carter-Wallace. I obtained this material from Carter-Wallace's R&D department. The "$Gold_{15}$ 108" notation identifies a formulation of colloidal gold particles of 15 nanometers (nm) average size coated with anti-hCG antibody called B-108; as I reported on page 014, attached as **Exhibit A**,

this formulation had an optical density (OD) of 15.03 units per milliliter ($u.ml^{-1}$) at 525 nanometer (nm) wavelength (OD_{525}). I obtained this gold conjugate from Carter-Wallace's R&D department. The "Cellulose acetate" notation refers to a commercial material that was available in my laboratory and which I used as one of the supports for the "dipstick" chromatograph gold assay. Cellulose acetate is an electrophoresis paper that I was testing as a wick and support. The "pregnazyme tris buffer" notation refers to a buffer solution I obtained also from Carter-Wallace's manufacturing department, lot # J735010, used at the time in assay products then marketed by Carter-Wallace under the trade name "Pregnazyme." I used this buffer as a diluent for the hCG mother solution (100 IU/ml) to prepare the "hCG standard" at 1 IU/ml. Glass fiber paper is a permeable material that absorbs liquid samples by capillary action on the fibers. It is a commercial material that was available in my laboratory and which I used as another type of support for the "dipstick' chromatograph gold assay, the other support being cellulose acetate. However, to obtain a rigid strip for the devices, I always affixed the glass fiber strips to "gel bond," a rigid material onto which glass fiber adhered easily. I obtained the "negative urine pool" identified as lot # 113J056 (which had been demonstrated to be free of hCG) from Carter-Wallace's manufacturing department, and I used it as diluent for both the "hCG standard" solution (1 IU/ml) and the "$Gold_{15}$ 108" mother solution of OD_{525} 15.03 $u.ml^{-1}$ to prepare the liquid samples having a concentration of hCG of 0, 50, 150 mIU/ml, and 1 IU/ml, and a concentration of "$Gold_{15}$ 108" of OD_{525} 1 $u.ml^{-1}$.

20. To perform the experiment recorded at page 014 of my notebook, attached as **Exhibit A**, I applied a formulation of anti-hCG antibody sensitized latex (B151-sensitized latex) on a portion of both a sheet of glass-fiber paper and on a portion of a sheet of cellulose acetate, with a Pasteur pipette, and allowed the formulation to dry on the sheets. I then mounted

the latex-embedded glass-fiber paper onto gel bond material for support. I cut the sheets into strips (about 5 x 7 (millimeters, mm)) which would fit into test tubes (10 mm in diameter). Each resulting strip of glass fiber on gel bond (see the four strips on the lower right of page 014) had a portion on which was embedded B151-sensitized latex beads. This portion, located about 1/2 inch from the lower extremity of the strip, constituted the test site. Each strip of cellulose acetate (see the two strips on the lower left of page 014) included a portion whereon was disposed the 151-sensitized latex beads located about one inch from the bottom of the strips. As a result, the B151-sensitized latex beads became immobilized on the glass-fiber paper, and on the surface of the cellulose acetate.

21. I continued the experiment by preparing several "diluents" for the gold conjugate by diluting the hCG standard with the negative urine pool to various concentrations of hCG, namely, 50 mIU/ml, 150 mIU/ml and 1 IU/ml (see the hand notation beside or below each strips affixed on page 014 attached as **Exhibit A**); the 0 IU/ml of hCG is the negative urine pool. I then diluted a mother solution of colloidal gold conjugate (OD_{525} 15.03 ml^{-1}) with the "diluents" to obtain liquid samples with a gold concentration of OD_{525} 1 ml^{-1} and 0 to 1 IU/ml of hCG. In particular, as noted in the middle of page 014, attached as **Exhibit A**, I mixed 16.63 microliters (ul) of $gold_{15}$ 108 mother solution with 233.37 µl of diluent to produce 250 µl of liquid sample. Next, I contacted the bottom portion of each glass-fiber strip with one of the four 250 µl of liquid samples, and each cellulose acetate strip into one of the two liquid samples. I observed the liquid samples with the gold conjugate contained therein wicked up the strips and passed into and through the test sites.

22. After ten minutes, as noted on **Exhibit A**, I inspected each strip for the presence of any color development and observed a color change at the test sites, i.e., turning pink to

red in color, against the background color of the strips. This color change, as I expected, was produced by the accumulation at the test sites of the colloidal gold conjugate in the "sandwich" complex comprising (antibody-coated latex/hCG/antibody-coated gold). I removed the test strips from the test tubes after 30 minutes, by which time the strips had become saturated with the liquid samples.

23. I recorded and preserved the results of this experiment on the lower half of attached **Exhibit A** under the heading "Results." I secured the actual four strips of glass fiber used in this experiment on the lower right corner of the page, and the actual two strips of cellulose acetate on the lower left corner of the page using adhesive tape. Beside or below each strip, I noted the concentration of hCG of the liquid samples used for that strip. I also wrote down the amount of liquid sample containing the colloidal gold conjugate/hCG mixture, i.e., 250λ (where λ is a shorthand for μl) used to wick up each strip.

24. This experiment proved that Dr. Charlton's dipstick concept worked. In particular, the dipsticks I made and used in this experiment constituted an actual reduction to practice of the device that Dr. Charlton described to me and which is now claimed in pending patent application serial No. 08/465,675. My use of these devices in carrying out the experiment constituted an actual reduction to practice of the method that Dr. Charlton described to me and which is now claimed in pending patent application serial No. 08/465,675. More specifically, the experiment produced at the test site on the strips, as is apparent on visual inspection of **Exhibit A**, a visible color change (pale pink for 50 mIU/ml of hCG to deep pink for 1 IU/ml of hCG). The change was a quantitative and qualitative indication of the presence of an analyte, namely, hCG. This color change is plainly visible on the photostatic copies of the

strips affixed to page 014, attached as **Exhibit A**, and on the originals.

25. After running this experiment, we sought to identify materials which might enhance and improve the speed and performance of the test. For example, after we performed the test described in attached **Exhibit A**, Dr. Charlton and I tried to improve the speed of the assay by testing different materials for the strips. We also conducted assays using this format with colloidal gold particles of different sizes, different hCG-specific antibodies, and different amounts of antibody-coated latex immobilized on the strips.

26. For example, attached **Exhibit E** is a true and correct copy of page 1608-025 of my laboratory notebook # 1608, except that the dates have been redacted. I have reviewed an unredacted copy of this exhibit and it bears dates that precede March 27, 1987. I handwrote this page as I performed the experiment described therein prior to March 27, 1987. Page 025, attached as **Exhibit E**, is entitled "b108 gold15 nm and 30 nm in chromatograph assay," and shows that I again performed the Easy Assay "dipstick" test format prior to March 27, 1987. I conducted the experiment described on this page to determine the effect on the test, if any, of different colloidal gold particle sizes. I carried out this experiment in the United States of America using "b108 $gold_{30nm}$," "b108 $gold_{15}$," "151 sensitized latex," "glass fiber paper," and "urine standards." I listed these materials on the top half of **Exhibit E**, under the heading "Materials."

27. The "b108 $gold_{30\ nm}$" notation in **Exhibit E** identifies a formulation of colloidal gold particles of 30 nm average size coated with hCG-specific antibody called B-108 which I obtained from Carter-Wallace's R&D department and had been previously made on a date prior to March 27, 1987, but which is redacted. The "b108 $gold_{15}$" notation identifies a formulation of colloidal gold particles of 15 nm average size coated with hCG-specific

antibody called B-108 which I also obtained from Carter-Wallace's R&D department and had been previously manufactured (as indicated) on a date prior to March 27, 1987, but which is redacted. Both conjugates, prior to lyophilization, had been suspended in a buffer solution specifically developed by Carter-Wallace's R&D department to stabilize the gold conjugates. As noted on the top of page 025, attached as **Exhibit E**, this buffer is composed of 0.1 M K_2PO_4, 1% bovine serum albumin (BSA), 4% polyvinylpyrrolidone (PVP), 0.02% polyethyleneglycol (PEG) and 0.1% NaN_3 having a pH 7.5. The "151 Sensitized latex" notation identifies a formulation of latex particles coated with another hCG-specific antibody called B-151. I prepared this antibody-latex formulation following the protocol developed by Carter-Wallace's manufacturing department which I reported on page 1608-015 of my notebook # 1608, a true and correct copy of which is attached hereto as **Exhibit B**, except that the dates on this exhibit have been redacted and are earlier than March 27, 1987. I have reviewed an unredacted copy of this exhibit and it bears dates that precede March 27, 1987. I applied this latex formulation on the glass fiber test strips following the protocol described on page 1608-024 of my notebook # 1608, a true and correct copy of which is attached hereto as **Exhibit D**, except that the dates have been redacted but are earlier than March 27, 1987. I have reviewed an unredacted copy of this exhibit and it bears dates that precede March 27, 1987. The "urine standards" notation identifies the liquid samples containing hCG at various concentrations which I prepared using the negative urine pool obtained from Carter-Wallace's manufacturing department as lot # 115J056.

28. To perform this experiment, as noted on the top of **Exhibit B**, I used the following materials: (1) 151 antibody purified on diethylaminoethyl ion exchange resin (Deae) at a concentration of 0.185 milligram per milliliter (mg/ml) in a phosphate buffer; (2) latex beads of 0.3 µm average size (lot # J644 DOM); (3) buffer solution 0.1 M of glycine buffer solution

(GBS) at pH 8.3, 0.1% NaN_3, 0.1% fatty-acid-free BSA (FAF BSA)(bovine albumin powder lot # x41401); (4) Tween® 20 (Sigma lot # 12F0299). I concentrated the 151 antibody in 0.1 M GBS of pH 8.3 to an optical density at 280 nm of 0.665 ml^{-1}. This corresponded to a concentration of antibody of 0.48 mg/ml. I listed these materials on the top half of page 015 shown in **Exhibit B** under the heading "Materials."

29. As noted on **Exhibit B**, I coated the latex beads with the hCG-specific antibody by mixing equal volumes of latex suspension and antibody solution to a total volume of 32 ml. I incubated the resulting mixture for five hours at 37 °C in a water bath with intermittent mixing every fifteen minutes. I further incubated the resulting mixture at 4°C for three hours with stirring every hour. I divided the resulting mixture in equal volumes into two tubes which I placed in the centrifuge operated at 3600 xG for twenty minutes. I then poured off 15 ml of the supernatants. The first attempt to resuspend the solids in 15 ml of 0.1% FAF BSA solution by sonication and vortex was unsatisfactory as it provided clumpy latex beads. As a result, I again placed the mixtures in the centrifuge, removed the supernatants, and added buffer solution to yield two good cloudy suspensions of antibody-coated latex. I left the suspensions overnight at 4°C. The following day, I placed the suspensions in the centrifuge at 4000 xG for forty minutes, removed the supernatants, and resuspended the solids, this time in 14 ml of the buffer solution. Afterward, I added 40 µl of 0.01% Tween® 20 to one of the latex samples.

30. I prepared the test strips by applying the formulation of b151-sensitized latex, prepared as described in page 015 (**Exhibit B**) and outlined above, to a sheet of glass-fiber paper as described on page 024 shown as **Exhibit D**. Page 024, entitled "Optimize latex coating procedure and volume for strip" is a record I made of the materials and procedure I

followed in preparing and optimizing the application of the "151 sensitized latex" on the strips. On the top half of page 024, I listed the materials I used in preparing those strips under the heading "Materials." I also made a drawing of the apparatus I used and which was composed of three parts: (1) a tubing connected to a vacuum line at one of its end and to a vacuum head, (2) the vacuum head in the form of a funnel with a perforated disk on its top, and (3) a gel bond template guide with a cut-out line.

31. To apply the latex to the glass fiber paper, I placed a sheet of glass fiber paper between the vacuum head and the template guide. I applied through the aperture of the template guide various volumes of B151-sensitized latex with a pipette: 10, 20, 50, 100 and 200 µl (see the middle of page 024, attached as **Exhibit D** where the notation λ is a shorthand for µl). I tested the sensitivity of the strips thus prepared with a mixture of hCG at 1 IU/ml and a conjugate of antibody B105 coated on 15 nm gold particles (b105-$gold_{15}$) which I prepared earlier and reported at page 1608-021 of my laboratory notebook #1608, a true and correct copy of which is attached as **Exhibit C** except that all the dates have been redacted. The results of this test can be seen at the bottom of page 024 under the heading "Results" where I have secured the strips with adhesive tape. Various shades of color developed at the site of application of the sensitized latex due to the formation of the sandwich complex antibody-coated latex/hCG/antibody-coated gold, from pale pink to deep pink as a function of (1) the concentration of hCG used (the five strips on the lower right), and (2) the amount of latex suspension applied (the five strips on the lower left). I recorded the amount of latex and the concentration of hCG used for each strip beside or below each respective strip.

32. Returning to the experiment I conducted and recorded at page 025 of my laboratory notebook and shown in **Exhibit E**, I

then prepared three sets of glass-fiber gel-bond strips with 151-sensitized latex applied on them. Also, I diluted a mother solution of hCG at 1 IU/ml with the negative urine pool to 25, 50, and 100 mIU/ml; the 0 IU/ml hCG concentration being the negative urine pool without addition of any hCG. Next, I reconstituted a sample of lyophilized b108gold$_{15}$ conjugate with 500 µl of one of the five hCG standard solutions and repeated this operation with other sample of lyophilized b108gold$_{15}$ conjugate with the other hCG standard solutions to provide a first series of liquid samples having b108gold$_{15}$ and hCG at a concentration of 0, 25, 50, 100 mIU/ml and 1 IU/ml. I repeated the same procedure with b108gold$_{30\ nm}$ to provide a second series of liquid samples having b108gold$_{30\ nm}$ and hCG at a concentration of 0, 25, 50, 100 mIU/ml and 1 IU/ml and b108gold$_{30\ nm}$. Then, as noted on **Exhibit E**, I transferred 250 µl of each of these liquid samples, each into a separate test tube (10x50 mm), and introduced into each test tube a gel-bond glass-fiber strip having b151-sensitized latex immobilized at the test site so as to contact the bottom portion of each strip with the respective liquid sample. This caused the liquid samples with the gold conjugate contained therein to wick up the strips and to pass through the test sites. I removed the strips after all of each liquid samples had absorbed onto the strips.

33. I recorded the results of this experiment on the lower half of **Exhibit B** by securing the strips used in the experiment on the page with adhesive tape. Below each strip, I recorded the concentration of hCG present in the liquid sample in which the strip was dipped. The images of the strips shown on **Exhibit E** are photostatic reproductions of the original test strips which I secured in the original notebook page. Three series are shown.

34. These experiments further confirmed the workability of Dr. Charlton's "dipstick" assay format prior to March 27, 1987. In particular, the dipsticks I made and used in these

experiments constituted an actual reduction to practice of the device that Dr. Charlton described to me and which is now claimed in pending patent application serial No. 08/465,675. My use of these devices in carrying out these experiments constituted an actual reduction to practice of the method that Dr. Charlton described to me and which is now claimed in pending patent application serial No. 08/465,675. More specifically, each series of strips attached to page 025 of my laboratory notebook, attached as **Exhibit E**, shows a dose response to hCG. In particular, the strips show an observable color signal at the test sites. The presence of color development at the test site of each strip provides a qualitative means of determining the presence of hCG in the liquid sample. A gradient of color intensity from no color or very pale pink for the 0 IU/ml hCG to deep pink for the 1 IU/ml hCG is observable on each series of strips shown on **Exhibit E**. This gradient of color intensity provides a quantitative means of determining the presence of hCG as the intensity of color increases with the concentration of hCG present in the liquid sample. The color development is due to the formation at the test sites of the sandwich antibody-coated latex/hCG/antibody-coated gold when hCG is present. The color gradient results from greater accumulation of gold particles at the test site at higher concentrations of hCG in the liquid samples.

35. In continuing the development of the "dipstick" assay format, I again practiced the assay prior to March 27, 1987, while experimenting to assess the maximum determinable concentration of hCG in a liquid sample using the dipstick assay format.

36. For example, **Exhibit G** is a true and correct copy of page 1608-036 of my laboratory notebook # 1608 except that the date has been redacted. I have reviewed an unredacted copy of this exhibit and it bears dates that precede March 27, 1987. I handwrote this page prior to March 27, 1987, to record the

procedure and results of an experiment entitled "High Dose Hook: 151 glass fiber paper". I conducted this experiment to determine the Easy Assay "dipstick" test's response to high concentrations of hCG (also termed "high dose hook"). As indicated on **Exhibit G**, I performed the experiment in the United States of America using "b108 $gold_{30nm,}$" "glass fiber paper," "151 SAS purified antibody," "151-sensitized latex," hCG standards, and "negative urine pool." I listed these materials on the top half of page 036 shown in **Exhibit G**, under the heading "Materials."

37. The "b108 $gold_{30\ nm}$" notation identifies a formulation of lyophilized colloidal gold particles of 30 nm average size coated with hCG-specific antibody called B-108 which I obtained from Carter-Wallace's R&D department. Prior to lyophilization, the conjugate was suspended in a buffer solution to stabilize the gold conjugate. As noted on **Exhibit G**, this buffer was composed of 0.1 M K_2PO_4, 1% bovine serum albumin (BSA), 4% polyvinylpyrrolidone (PVP), 0.02% polyethyleneglycol (PEG), 0.1 M boric acid, and 0.1% Ficoll having a pH 7.5. The "151 SAS purified" notation identifies another hCG-specific antibody called B-151 purified by precipitation using saturated ammonium sulfate. The "151 Sensitized latex" notation identifies a formulation of latex particles coated with hCG-specific antibody B-151. I obtained this latex formulation from Carter-Wallace's R&D department. The "hCG standards" notation identified the commercial hCG solution having a concentration of 250 IU/ml, lot #115F-0187, purchased by Carter-Wallace from Sigma Chemical Company. The "negative urine pool" identifies the liquid medium used to prepare urine samples having various concentration of hCG by dilution of the hCG standard solution. These urine samples are used to prepare the liquid samples by reconstituting the gold conjugate. I obtain the negative urine pool from the manufacturing department as lot # 115J056.

38. To perform this experiment, as I noted on page 036, attached as **Exhibit G**, I first prepared two sets of glass fiber

strips: 1) one with purified antibody B-151 embedded in a portion of the glass fiber strip located about half an inch from the bottom of the strip; and 2) the second with 151-sensitized latex embedded in a portion of the glass fiber strip also located about half an inch from the bottom of the strip. I then prepared the urine samples, as I noted on page 036, attached as **Exhibit G**, by diluting the hCG standard solution from 250 IU/ml to 25, 50, and 100 mIU/ml and 1 and 100 IU/ml. For the 0 IU/ml sample, I used the negative urine pool. Next, I reconstituted 12 samples of gold conjugate, two sets of six, one of each set with a separate urine sample thus making two sets of liquid samples each having the same concentration of gold conjugate and a varying concentration of hCG from 0 to 100 IU/ml. As I indicated on page 036, attached as **Exhibit G**, under the heading "procedure," I then transferred 250 µl of each liquid sample in one of twelve clean glass tubes (10x50 mm) to which I added the two sets of glass fiber strips. This caused the liquid samples with the gold conjugate contained therein to wick up the strips and to pass through the test sites. I observed the strips after ten minutes.

39. The results of the experiment recorded at page 036, attached as **Exhibit G**, can be seen at the bottom of that page where I secured the two sets of strips I actually used in this experiment using adhesive tape. On the bottom left, I affixed the set using glass fiber with B-151 antibody embedded at the test site. On the bottom right, I affixed the set using 151-sensitized latex at the test site. Below the set using 151-sensitized latex and close to each strip, I recorded the concentration of hCG in the liquid sample used on that strip. However, I also noted on the right side of that set that I have in fact used the sample with 25 mIU/ml of hCG on the strip marked for 100 mIU/ml and vice-versa.

40. The experiment recorded at page 036, attached as **Exhibit G**, which I performed prior to March 27, 1987, again

proved the dipstick concept to be a working assay format. In particular, the dipsticks I made and used in this experiment constituted an actual reduction to practice of the device that Dr. Charlton described to me and which is now claimed in pending patent application serial No. 08/465,675. My use of these devices in carrying out the experiment constituted an actual reduction to practice of the method that Dr. Charlton described to me and which is now claimed in pending patent application serial No. 08/465,675. More specifically, the results clearly indicate a test format specific to the presence of hCG by showing a dose response to hCG, provided that antibody-coated latex is used at the test site. This is observable by visual inspection of the color developed at the test site of each strip (pale pink for 25 mIU of hCG to deep pink for 1 IU of hCG) against the color background of the strip (white). This experiment also demonstrates the need to immobilize effectively the antibody specific for hCG at the test site in order to observe a color development. This is shown by the absence of color development in the set of strips using only B-151 antibody not coated on latex beads at the test site. Drying the 151-antibody directly on the glass fiber was insufficient to immobilized effectively the antibody at the test site. This experiment further demonstrates that to observe a color development at the test site, the second antibody to hCG has to be immobilized so that accumulation of the gold conjugate as a sandwich complex antibody-gold conjugate/hCG/antibody-coated latex may occur. Also, the color gradient is indicative of an assay format sensitive to varying concentration of hCG and provides a quantitative means of determining the presence of hCG. I observed the lower limit of detectability in this experiment at 25 mIU/ml of hCG.

41. The results of the experiments I performed were regularly presented at meetings at Carter-Wallace. The content of these meetings was then recorded and summarized in memoranda circulated among various persons at Carter-Wallace. For

example, prior to March 27. 1987, Dr. Charlton and I presented the Easy Assay "dipstick" format and the procedure used to determine the presence of hCG in a urine sample using the dipstick devices I fabricated in the lab at one of these meetings. A summary of that presentation is recorded under the section titled "'Chromatograph' Direct Concept Status" in a memorandum which was circulated prior to March 27, 1987, a true and correct copy of which is attached as **Exhibit J** except that the dates have been redacted. I have reviewed an unredacted copy of this exhibit and it bears dates that precede March 27, 1987. My name appears in the list of attendees as M. Verbanic, which is my maiden name, and also in the distribution list. I attended the meeting referred to in the memo and received a copy of the memo on or around the time of its date. Under subheading 1., the summary indicates the assay to be workable for a read in two to five minutes using a conjugate of gold 30 nm with hCG-specific antibody b108, a urine sample and a membrane/stick. Under subheading 2., the summary indicates the use of pre-coated membrane with latex in form of bars. This is a short description for hCG-specific antibody B151-sensitized latex immobilized on the strip.

42. Dr. Charlton also presented to me the Easy Assay "pee-on" test concept prior to March 27, 1987. Under his direction, I practice the Easy Assay "pee-on" test format prior to March 27, 1987.

43. For example, attached **Exhibit F** is a true and correct copy of page 1608-33 (page 033) of my laboratory notebook #1608, entitled "Lyophilize $gold_{15}$-105 and $gold_{30}$-108 into disks," except that the dates have been redacted. I have reviewed an unredacted copy of this exhibit and it bears dates that precede March 27, 1987. I handwrote this page to recorded the procedure and results of the Easy Assay "pee-on" test format prior to March 27, 1987. I performed this experiment in the United States of America to test the ability of various types of solid support to release the lyophilized conjugate in the flow of a

liquid sample. I listed the materials used in this experiment on the top half of the page under the heading "Materials."

44. I first prepared disks of various types of solid support by punching out two circular portions of each sheet of the following solid supports which are commercially available through Whatman under the following designations which I listed in **Exhibit F** under the heading "materials": (1) GF/D; (2) D801; (3) D28; (4) 17 Chr; (5) unknown designation represented by the question mark (?) which Dr. Charlton and I believed to be similar to the actual GF/D. Next I lyophilized $gold_{15}$-105 and $gold_{30}$-108 onto the disks. I then mounted one of each pair of disks on gel bond support adjoining 1) a strip of glass-fiber paper containing 151-sensitized latex embedded at the test site, such that at least a portion of the disk would be coplanar with the strip for each device, and 2) another absorbent material made of either cigarette filter or blotter paper. I pipetted a liquid sample onto the disks until the disks and the strips had been saturated with the liquid sample. I recorded the results of this experiment on page 033 of my notebook #1608, attached as **Exhibit F**, by securing the series of spare disks with adhesive tape and also securing below each spare disk the corresponding device made of the strip and the disk with adhesive tape. Below each disk I noted the type of solid support used to lyophilized the conjugate.

45. This experiment showed the feasibility of the "pee-on" assay format. In particular, the dipsticks I made and used in this experiment constituted an actual reduction to practice of the device that Dr. Charlton described to me and which is now claimed in pending patent application serial No. 08/465,675. My use of these devices in carrying out the experiment constituted an actual reduction to practice of the method that Dr. Charlton described to me and which is now claimed in pending patent application serial No. 08/465,675. More specifically, the devices that I secured to my laboratory notebook at page 033,

shown in **Exhibit F**, showed that the gold-antibody conjugates can be dried out on a solid support, then reconstituted in the course of the assay by the moving front of a liquid sample moving therethrough, and entrained toward and through the test site containing a binder for the analyte. This result can be observed by comparison of the color on the disks before (spare disks), and after (disks mounted beside the test strips), the application of the liquid sample, and by the appearance of a light pink color at the test site due to binding of the conjugate at the test site. At the bottom of page 033 I noted that "the dried dot conjugate, in same format, would not redissolved or flow properly." This refers to my observation that, in an experiment I conducted earlier, gold conjugate lyophilized onto hydrophobic surfaces such as gel bond could not be released properly.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: October 7, 1997

Margaret M. Mazzeo

Margaret M. Mazzeo

PATENT
Atty. Docket No. CWP-012CN
(1451/2)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): Charlton et al.

SERIAL NUMBER: 08/465,675 GROUP NUMBER: 1817

FILING DATE: June 6, 1995 EXAMINER: C. A. Spiegel

TITLE: Test Device and Method For Colored Particle Immunoassay

DECLARATION OF NEIL W. MILLER

I, NEIL W. MILLER, declare:

1. I am currently employed at Wyeth-Ayerst in St. Davids, Pennsylvania as a Package Engineer since 1993 and reside at 415 Society Place, Newtown, Pennsylvania 18940.

2. I received a Bachelor of Science Degree in Industrial Design from the Philadelphia College of Art, in Pennsylvania, in 1972.

3. I began working at Carter-Wallace, Inc. ("Carter-Wallace"), Carter R&D Division, in Cranberry, New Jersey, U.S.A. in the summer of 1984 as a Package Designer. In that position, my job responsibilities included designing product lines and management of laboratory personnel. Thereafter, I transferred to the Diagnostic Division of Carter-Wallace in East Windsor, New Jersey, where I was promoted to the position of Senior Bioengineer. In that position my work responsibilities included new product development of diagnostic assays. Later, I transferred to Carter Products R&D division of Carter-Wallace. Thereafter, I was promoted

to the position of Package Designer. I remained in that position until 1992. As a Package Designer, my work responsibilities included evaluation of components for the current Carter-Wallace line of products.

4. In particular, during the course of my employment at Carter-Wallace, I worked on the development of new diagnostic products. Specifically, I worked on a diagnostic product referred to within Carter-Wallace as the "Easy Assay" project.

5. Initially, the Easy Assay project contemplated several different test formats. In particular, products being developed within the Easy Assay project embodied: (1) "platform"; (2) "dipstick" (also called "chromatostrip" or "chromatostick"); and (3) "pee-on" formats.

6. Prior to March 27, 1987, Dr. David Charlton conceived of the Easy Assay "dipstick" and "pee-on" formats as chromatographic tests using colloidal gold labeled antibody, and a capturing antibody immobilized within a zone on a strip of membrane material. He discussed his ideas with me, and together, in the United States, we developed test devices and methods including specially designed housings for test strips for carrying out "dipstick" and "pee-on" assays.

7. Dr. Charlton described the "pee-on" concept to me, and following our discussion, I prepared a drawing of a prototype for a "pee-on" device. Afterward, Dr. Charlton and I presented the pee-on device with the drawing of the prototype at one of the Gold Sol Membrane Meetings held at Carter-Wallace in New Jersey prior to March 27, 1987. The content of this meeting was summarized in a memorandum and circulated to "Distribution", including me, from Ms. M.

Goddard prior to March 27, 1987, a true and correct copy of which is attached hereto as Exhibit I, except that all the dates appearing thereon have been redacted. I have reviewed the unredacted copy which bears a date that precedes March 27, 1987.

8. As can be seen in Exhibit I, my name and the name of Dr. Charlton appear on the list of attendees and on the memo distribution list. I attended the meeting referred to in this memo and received a copy of this memo on or shortly after the time of its date. On page two of Exhibit I, under the heading "Concept Presentation: 'Easy Assay' Direct Sample Application Test," the memo relates an accurate summary of the content of the discussion held at the meeting concerning the presentation of the "pee-on" concept and represents a good description of the "pee-on" concept as it was presented to me by Dr. Charlton. In particular, under subheading 2., the memo describes that "the device wicks the sample through pre-filled conjugate zone and both control and patient reading zones."

9. Page three of Exhibit I shows the drawings of the "pee-on" concept I made prior to March 27, 1987. These drawings show two figures: one depicting the outer casing of the device with an absorbent portion exposed to the outside; and the other depicting the strip contained within the device having a portion for the immobilization of the reagents, such as the antibody-coated latex, and another portion for the conjugate, analyte-specific antibody conjugated to a gold particle.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable

by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: OCT. 7, 1997

Neil W. Miller

PATENT
Atty. Docket No. CWP-012CN
(1451/2)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Charlton et al.		
SERIAL NUMBER:	08/465,675	GROUP NUMBER:	1817
FILING DATE:	June 6, 1995	EXAMINER:	C.A. Spiegel
TITLE:	Test Device and Method for Colored Particle Immunoassay		

DECLARATION OF KEVIN B. CLARKE

I, KEVIN B. CLARKE, declare:

1. I am Patent Counsel for Carter-Wallace, Inc. at 1345 Avenue of the Americas, New York, NY 10105.

2. I have been Patent Counsel for Carter-Wallace since 1970.

3. I have reviewed the original documents of which attached Exhibits A-L are photostatic copies.

4. Specifically, Exhibits A-G are true and correct copies, except that all the dates appearing thereon have been redacted, and are before March 27, 1987, of certain pages of a laboratory notebook, #1608, originally issued, maintained, and filled out by Ms. Margaret Verbanic during the course of her employment at Carter-Wallace, Inc. Ms. Verbanic was under standing instructions as a worker in laboratories maintained by Carter-Wallace, Inc. to compose and maintain this and other notebooks as a means to preserve a substantially contemporaneous record of her daily activities in her laboratory at

Wampole Division. This notebook was created, maintained and, since its completion, has been kept as part of the corporate records in the regular course of business at Carter-Wallace, Inc.

5. Exhibits H-L are true and correct copies, except that all the dates appearing thereon have been redacted, of memoranda created by various Carter-Wallace personnel in the regular course of their employment to preserve a record of discussions, recommendations and decisions taken concerning certain specific Carter-Wallace research and development projects. These memoranda were generated, and since generated have been kept, at Carter-Wallace, Inc. as part of the corporate records in Carter-Wallace's regular course of business. I hereby declare that these documents are copies of documents which have been present in the files of Carter-Wallace, Inc. since shortly after their creation, prior to March 27, 1987, and that they were retrieved from the files of Carter-Wallace by me or persons under my direction in response to my requests for the location of documents corroborating conception or reduction to practice of the subject matter of the above-referenced application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 10-6-97

Kevin B. Clarke

KEVIN B. CLARKE

EXHIBIT A

TE REDACTED	EXPERIMENT NO. 11	CROSS REFERENCE NB-	BOOK NO. Nº 1608-014	PAGE

RPOSE Test feasability of Chromatograph Gold Assay

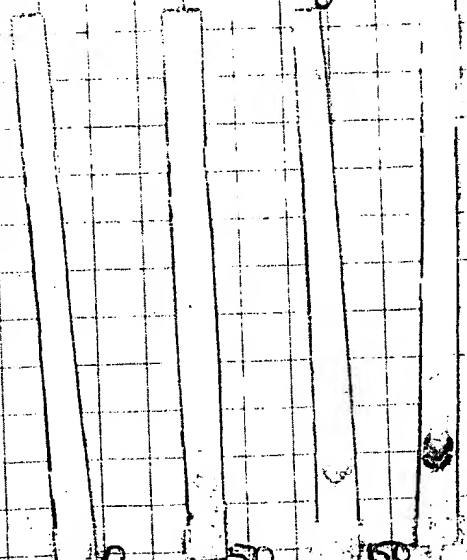
Materials: 151 - sensitized latex
gold. 15 108 OD 15.03

hCG standards 100 IU/ml
cellulose acetate - diluted in pregnazyme
tris buffer J735010 1:100 to
yield 1 IU/ml, this used as
diluent for gold, (1:15.03) dilution
yield 1 OD gold

glass fiber on gel bond support - standard
diluted in negative urine pool 113Jo8
these are used for gold conjugate dilution

gold dilution - 233.37 diluent
16.63 ml gold - 108

Results: Simultaneous assay - results visible after
10 min - removed after 30



0 IIU

standards in pregnazyme
J735010

250 gold 108 in standards
prepared in neg urine pool

SIGNED M. Lorraine
WITNESSED BY

DATE REDACTED
DATE

EXHIBIT A

DATE [redacted]	EXPERIMENT NO.	CROSS REFERENCE NB-	BOOK NO. Nº 1608-014	PAGE

PURPOSE Test feasability of Chromatograph Gold Assay

Materials: 151 - sensitized latex (binder for the analyte) gold. is 108 OD 15.03 (conjugate)

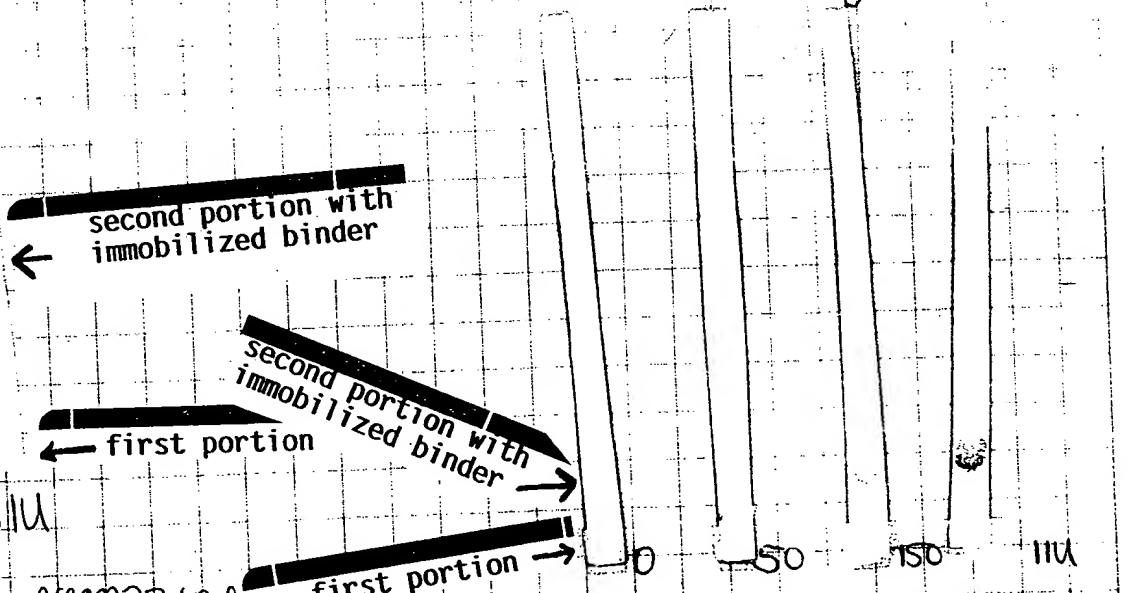
hCG standards 100 IU/ml (binder for the analyte)

cellulose acetate (colored particle) — diluted in pregnazyme tris buffer J735010 1:100 to yield 1 IU/ml, this used as diluent for gold (1:15.03) dilution yield 100 gold

glass fiber on gel bond support - standard diluted in negative urine pool 1:30 these are used for gold conjugate dilution

gold dilution - 233.37 diluent
16.63 ml gold - 608

Results: simultaneous assay - results visible after 10 min. - removed after 30



Standards in pregnazyme J735010

250x gold 108 in standards prepared in neg urine pool

SIGNED M. Vitanova BA: [redacted]
WITNESSED BY DATE

EXHIBIT B

DATE	EXPERIMENT NO.	CROSS REFERENCE	BOOK NO.	PAGE
REDACTED	12	NB-CA 1604-017	№ 1608-015	

PURPOSE Prepare 151 Sensitized Latex (.3μ)

Materials: 151 antibody Deae purified
185 mgs/ml in PO4 buffer
concentrated into .1M GBS pH 8.3
OD 280 = .665 → .48 mgs/ml

latex .3μ (5644 Dow)

.1M GBS pH 8.3 .1% NaN3 .1%
fatty acid free BSA
(Bovine albumin powder lot# 41401)

tween 20 (Sigma) lot# 12F0299

Procedure: mix equal volumes latex suspension and antibody solution — total volume 32mls

Incubate 2hrs 37°C (H2O bath) should mix every 15 min
incubated 3hrs — mixing every 15 min after 2nd hour

Incubate 3hrs @ 4°C — stirring every hour.

Split into two tubes in centrifuge — centrifuge 3600 xG for 20 minute = 3600RPM in Sorvall
pour off supernatant (note volume) 15mls — resuspend in 15 mls GBS .10% FAF BSA — to resuspend sonicated and vortex — still clumpy centrifuge again do before — super very cloudy — latex not brought down — leave over night 4°C centrifuge again 4000 xg 40 min (3800 RPM)

Resuspend in 14mls GBS .1% NaN3 FAF BSA. to one add .01% tween 20 = 40ml

SIGNED M. Verhaeve DATE REDACTED

WITNESSED BY DATE

EXHIBIT C

DATE	EXPERIMENT NO.	CROSS REFERENCE	BOOK NO.	PAGE
REDACTED	17	NB- 1608-006,007	Nº 1608-021	

PURPOSE To prepare b105 - gold 15 nm conjugate

Materials: gold 15nm (EY Laboratories lot # 040718)
b105 Antibody CA
diluted & dialyzed in 5mM borate pH 6.8
OD_{280} @ 1:50 = .203

$\frac{.203}{1.4} = (.145) \times 50 = 7.25$

5mM borate (boric acid pH 6.8 (mD))
.1M PO_4 pH 7.5 containing
1% PVP, .02% PEG, 1% BSA .1% NaN$_3$
above buffer containing 15% sucrose

Procedure: 50mls gold 15 — need 50mls 105 in borate at 10ugs/ml — 10ug/ml x 50 = 500ug / 7.25ugs/ul = 68.97uls
50mls - 68.97 = 49.931uls borate buffer

mix — wait 2min, add 2mls 1% PEG
spin gold through 15% sucrose cushion for 1hr at 24,000 RPM 16°C
Aspirate supernatant — resuspend in 1/100 x Vol of gold — min 5mls
add small aliquots — mix — transfer to next tube
OD_{525} (1:50) = .115 ⟶ x dilution factor = 5.75
total volume = 70mls

Lyophilization: 7.0 mls x 5.75 = 40 mls at 1 OD
40 / 5.75 = 6956.5 ul gold + 33043 stable buffer
OD = 1.092 + 500ul gold and 2375 buffer

Continue dilution to 1.0
final OD_{525} = 1.062
aliquot 25ul 10x50mm tube
yield 1179 tubes

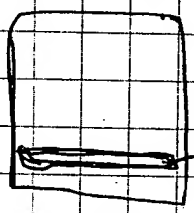
SIGNED M. Verbanic DATE REDACTED
WITNESSED BY DATE

EXHIBIT D

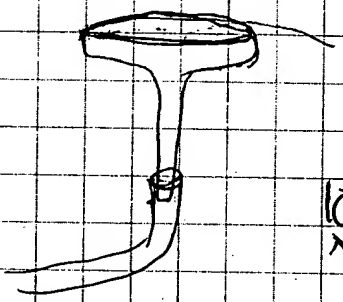
DATE	EXPERIMENT NO.	CROSS REFERENCE	BOOK NO.	PAGE
REDACTED	20	NB-	Nº 1608-024	

PURPOSE: Optimize latex coating procedure and volume for strip

Materials: 15% sensitized latex 1608-015
gel bond paper — with line cut out to act as template for vacuum device



whole

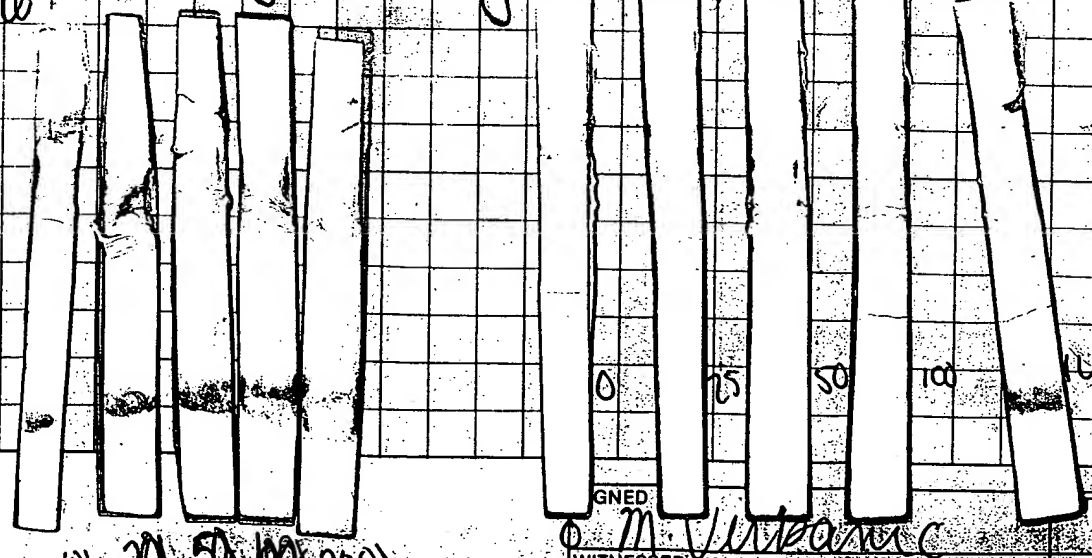


place glass fiber paper on lid vacuum device — pipet latex in various volumes
10λ, 20λ, 50λ 100, 200λ

Test latex in chromatograph assay at 1 hr Establish latex coat several strips - check sensitivity

Results:
cong 6105-gold 15 1608-02

15% latex



10λ 20λ 50λ 100 200λ

SIGNED: M. Urbanic
DATE: REDACTED
WITNESSED BY:
DATE:

EXHIBIT E

DATE	EXPERIMENT NO.	CROSS REFERENCE	BOOK NO.	PAGE
REDACTED	21	NB.	Nº 1608-025	

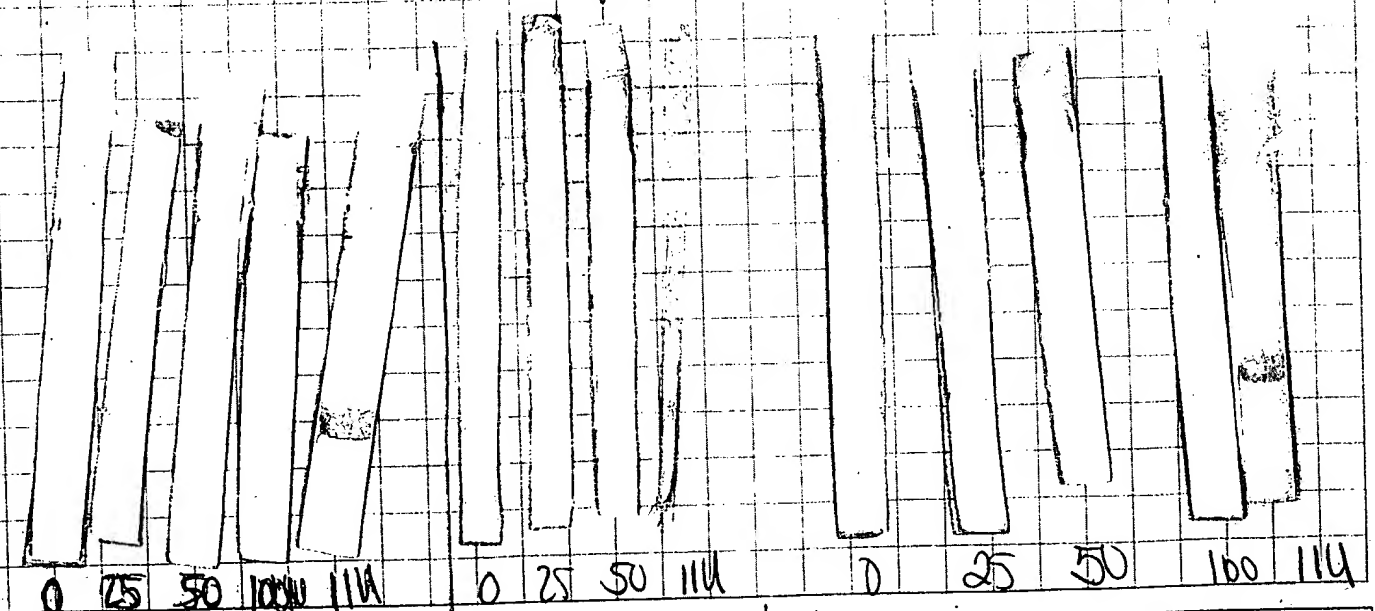
PURPOSE b108 gold 15nm and 30nm in chromatograph assay

Materials: (b108 gold 30nm (REDACTED BND) pH 7.5
1%MEPOG, 1%BSA, 4% PVP, .02% PEG, .1% NaN_3
(b108 gold 15 (REDACTED BND) pH 7.5

15l sensitized latex - applied to glass fiber paper as described on page 024 of this notebook

urine standards prepared in neg urine pool 15T056
IU → 100, 50, 25, 0 mIU hCG

Procedure: reconstitute gold conj - 15 or 30 in hCG standard 500 ul - Mix transfer 250 ul volume into clean 10x50mm test tube gold reagent strip w/ absorbant pad attached. Incubate until all fluid is absorbed.



thin strips

SIGNED M. Verbanic DAT REDACTED
WITNESSED BY DATE

EXHIBIT E

DATE	EXPERIMENT NO.	CROSS REFERENCE	BOOK NO.	PAGE
REDACTED	21	NB.	№ 1608-025	

PURPOSE b108 gold 15 and 30 nm in chromatograph assay

binder for the analyte

colored particle

Materials: (b108 gold 30 nm (REDACTED BND) pH 7.5
conjugate
(b108 gold 15 (REDACTED BND) pH 7.3

binder for the analyte

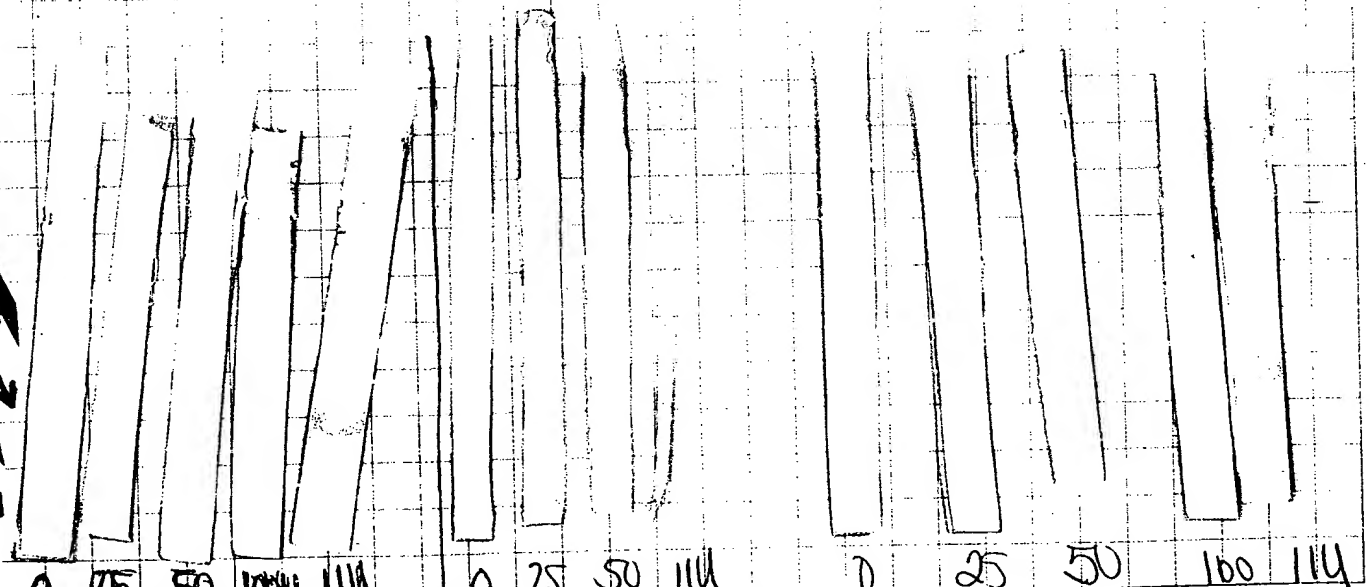
151 sensitized latex - applied to glass fiber paper as described on page 024 of this notebook

urines standards prepared in neg urine pool 115J056
hCG → 100, 50, 25, 0 mIU hCG

Procedure. reconstitute gold conj - 15 or 30 in hCG standard 500 ul — mix; transfer 250 ul volume into clean 10x50mm test tube; add reagent strip w/ absorbant pad attached. Incubate until all fluid is absorbed.

second portion with immobilized binder

first portion



thin strips

SIGNED M. Verbanic DAT REDACTED
WITNESSED BY DATE

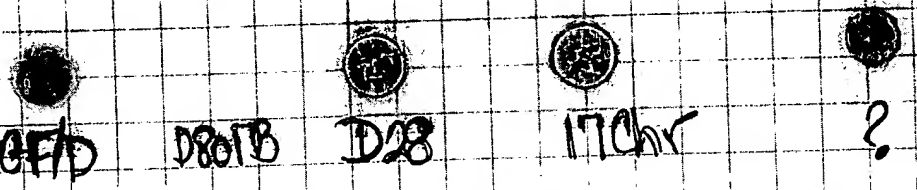
EXHIBIT F

DATE	EXPERIMENT NO.	CROSS REFERENCE	BOOK NO.	PAGE
REDACTED	29	NB-	Nº 1608-033	

PURPOSE Lyophilize gold 15-105 and gold 30-108 into disks

Materials: Whatman paper - punched out disks

GF/D
D801B
D28
17Chr
? ~ actual GF/D paper



2 = absorban

Chromatograph prototype: absorbant 1 = gig
absorbant 2 = blotte
paper

note: dry dot conjugate in some format would not redissolve and flow properly

SIGNED M. Vukanic
WITNESSED BY

DAT. REDACTED
DATE

EXHIBIT F

DATE	EXPERIMENT NO.	CROSS REFERENCE	BOOK NO.	PAGE
[redacted]	29	NB.	№ 1608-033	

PURPOSE Lyophilize gold 15-105 and gold 30-108 into disks

Materials: Whatman paper - punched out disks

binder for the analyte

colored particle

GF/D
D80 B
D28
17 Chr
? ~ actual GF/D paper

conjugate

absorbant 1

first portion

second portion with immobilized binder

Chromatograph prototype: absorbant 1 = glass fiber
absorbant 2 = blotting paper

Note: dry dot conjugate in some format would not redissolve and flow properly

SIGNED M. Vukanic
WITNESSED BY

DAT. [redacted]
DATE

EXHIBIT G

DATE [redacted]	EXPERIMENT NO. 38	CROSS REFERENCE NB-	BOOK NO. Nº 1608-036	PAGE

PURPOSE High Dose Hook: 151 glass fiber paper

Materials: 0108 gold 30nm
.1M KPO4 pH 7.5 1% BSA 4% PVP
.02% PEG, 1% NaN_3, 1M boric acid
1% Ficoll [redacted] BN

glass fiber paper w/ 10 ul 151 SAS purified 1556-085

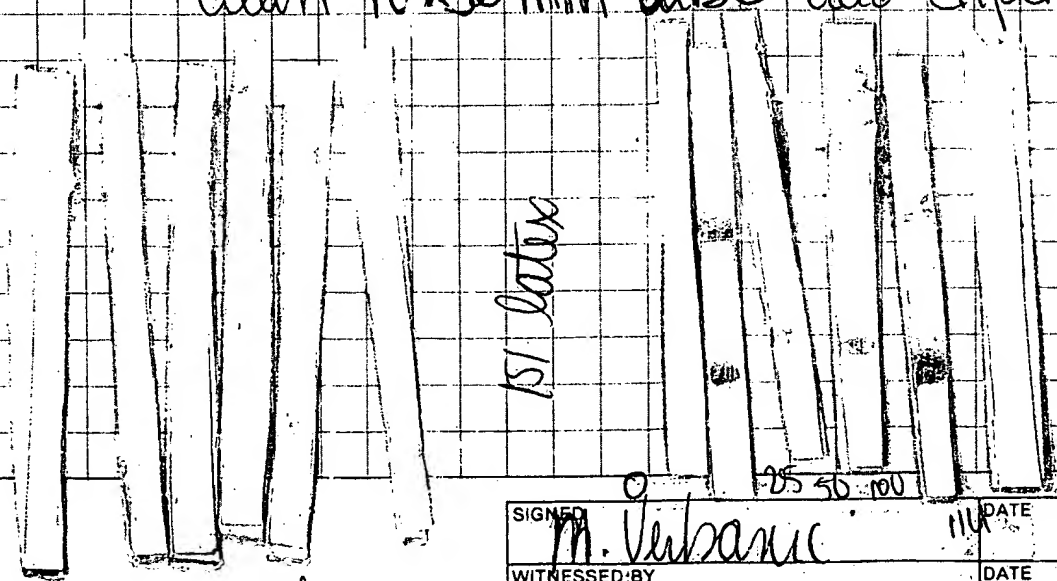
. glass fiber paper: (Whatman) GF/A

151 sensitized latex ([redacted] [redacted]

hCG standards in negative pool 115J056
using crude hCG Sigma lot# 115F-0187
250 IU/ml

	hCG	urine
100 IU	= 400 ul	+ 600 ul
1 IU	= 10 ul	+ 990 ul
100 mIU	= 100	+ 900
50	= 50 ul	+ 950
25	25 ul	+ 975 ul
0	neg urine pool	

Procedure: reconstitute gold conj – transfer 250 ul to clean 10×50 mm tube add dipstick / results 10 min



SAS 151 on glass fiber

Note: standards reversed 25 & 100 mIU

100 IU

SIGNED M. Verbanic	DATE [redacted]
WITNESSED BY	DATE

EXHIBIT G

DATE [redacted]	EXPERIMENT NO. 38	CROSS REFERENCE NB-	BOOK NO. Nº 1608-036	PAGE

PURPOSE High Dose Hook: 151 glass fiber paper

[redacted]

Materials: binder for the analyte — BIOS gold 30nm — colored particle

conjugate

.1M KPO4 pH 7.5 1% BSA 4% PVP
.02% PEG 1% NaN3 .1M boric acid
1% Ficoll [redacted] BN.

glass fiber paper w/ 10ul 151 SAS purified 1556-085

glass fiber paper: (Whatman) GF/A

151 sensitized latex ([redacted] [redacted]

binder for the analyte

hCG standards in neg urine pool 115J056
using crude hCG Sigma lot# 115F-0187

250 IU/ml — hCG — urine

100 IU	=	400 ul	+ 600 ul
1 IU	=	10 ul	+ 990 ul
100 mIU	=	100	+ 900
50	=	50 ul	+ 950
25	=	25 ul	+ 975 ul
0	=	neg urine pool	

Procedure: reconstitute and conj — transfer 250 ul to clean 10 x 75 mm tube add dipstick / results 10 min

second portion with immobilized binder

151 latex

first portion

note: standards reversed 25 & 100 mIU

0 25 50 100 1IU 100 IU

SAS 151 on glass fiber

SIGNED M. Verbanic DATE [redacted]

WITNESSED BY DATE

EXHIBIT H

MEMORANDUM

TO: Distribution

FROM: Melinda Goddard MG

DATE: REDACTED

SUBJECT: GOLD SOL MEMBRANE MEETING - REDACTED

Attendees: R. Barrish, D. Charlton, M. Goddard, R. McPartland, N. Miller, W. Wallen

Background

Dr. McPartland reviewed the events of previous meetings wherein a test format for Carter, International, and Wampole had been discussed.

The Abbott Testpack™ device, packaging, and unique "plus/minus" endpoints were presented as the state-of-the-art in professional membrane test formats.

Colloidal Gold Concepts

1. Carter has reporteldy shown feasibility for the conversion of Answer® Plus to gold sol, resulting in a 30-minute, 50 mIU test.

2. Additional work has been done in Carter Research based on lyophilized conjugate which is reconstituted with the urine sample (in a tube) into which a "wicking" strip is placed then read at 5 minutes for color development with as little as 25 mIU hCG/ml. They have been working toward a "stick" concept which could be held by the patient to eliminate conventional collection.

3. Wampole R&D has been working toward a 3-well format which could match the Abbott test in endpoint function (result and reagent control, plus a specimen control).

4. A "spoon-like" version of the Carter concept was discussed as a possible way to eliminate the tubes and sample pipets from the test.

5. Subcontracting of the "device" manufacturing was anticipated with the exception of the latex-loading step.

Prototype Development

1. Neil Miller would begin this process by drawing the product concepts.

2. Thermoform plastics and other materials such as foil-laminated papers or plastics were discussed for "in-house" prototype production.

3. It was also recommended that the R&D Departments cross-check with Nick Tabolinsky and Nick Fruscione to be completely informed relative to vendor status.

4. This project must reach production status within two months to meet corporate goals.

Follow-Up Meeting: REDACTED **at 9:30 a.m. in the Wampole Conference Room, Cranbury.**

Agenda: Review/Discuss Preliminary Concept Drawings

MG:ak

Distribution

C. Agius
R. Barrish
J. Bridgen
D. Charlton
A. Chizmadia
S. Christoff
J. Dekis
P. Freidenreich
R. Herzog
L. Isbrandt
P. Konanez
J. Kraeutler
R. McPartland
N. Miller
S. Riggio
B. Shears
N. Tabolinsky
C. Utz
W. Wallen

REDACTED

EXHIBIT I

MEMORANDUM

TO: Distribution

FROM: Ms. M. Goddard

DATE: REDACTED

SUBJECT: GOLD SOL MEMBRANE MEETING - REDACTED

Attendees:

C. Ageis
R. Barrish
J. Bridgen
D. Charlton
M. Goddard
A. Howell
P. Konanez
J. Kraeutler
R. McPartland
N. Miller
N. Tabolinsky
W. Wallen

Prototype Production: "Easy Assay" Platform Concept

Neil Miller presented a prototype with the following characteristics:

1. Thermoform construction with foil overlay/label on one side (top).
2. Foil peels off to run test.
3. Sides fold down to make "platform."
4. Hydrophobic material is on top with 3-wells and snap-fits into base over porex and membrane/latex.
5. The concept could require pipetting of urine and a colloidal gold conjugate reagent lyophilization, reconstitution, and delivery system.

Production Discussion

1. Latex loading could either preceed or follow device assembly.
2. Internal or external manual or automated systems could be employed.
3. Snap-fittings or adhesives could be evaluated.
4. Latex could be pipetted or printed.
5. None of these issues could be addressed without proceeding to prototypes: 100-200 prototype devices were targeted for availability by REDACTED to initiate stability studies, clinicals, and 510(k) data. To achieve this, R&D made a commitment to provide raw materials by REDACTED .

Concept Presentation: "Easy Assay" Direct Sample Application Test

A second concept was presented with the following characteristics:

1. The device could be sampled directly by the patient or from a urine cup.

2. The device "wicks" the sample through pre-filled conjugate and both control and patient reading zones.

3. This concept would create a single-step result for the user by eliminating urine pipetting and separate conjugate delivery systems.

4. While this was a most appealing concept, both designs must be pursued due to as yet spending feasibility of the latter design.

A follow-up meeting will take place on REDACTED at 1:30 p.m. in the Wampole Conference Room in Cranbury.

M. Goddard/ak

MG:ak

Attachments: 2

Distribution

C. Agius
R. Barrish
J. Bridgen
D. Charlton
A. Chizmadia
J. Dekis
P. Freidenreich
R. Herzog
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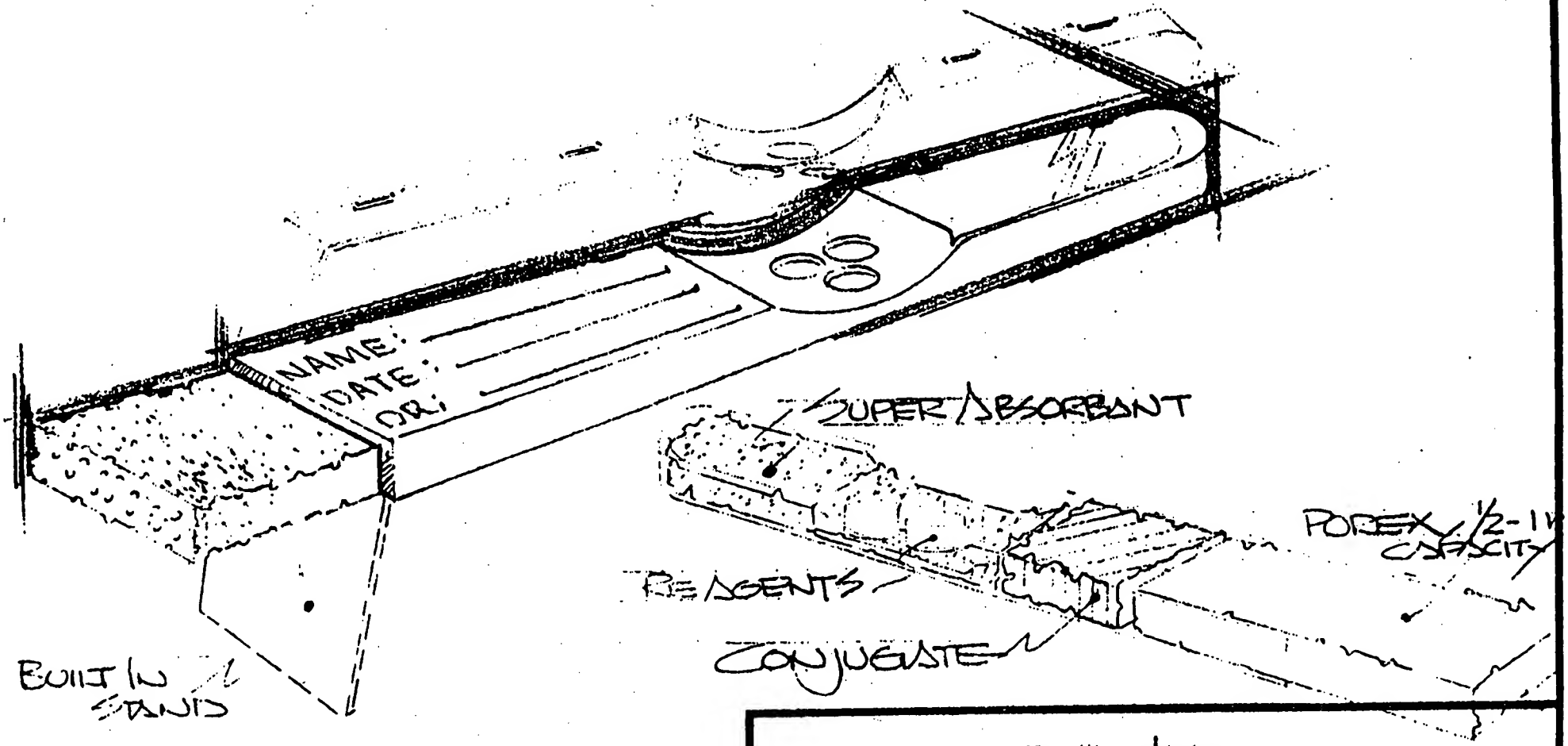
J. Kraeutler
R. McPartland
N. Miller
S. Riggio
B. Shears
N. Tabolinsky
C. Utz
W. Wallen

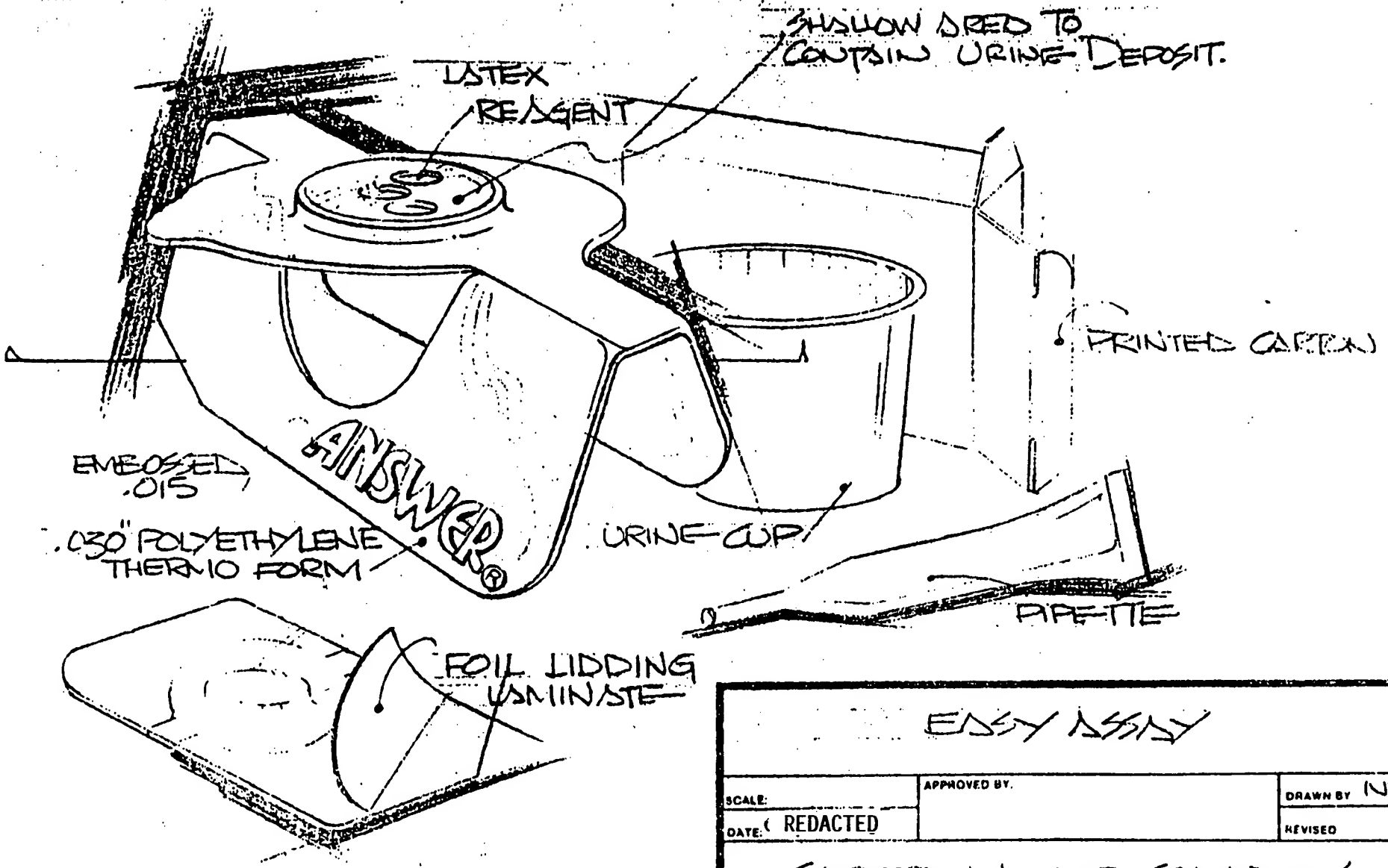
REDACTED



NO URINE CUP/NO PIPETTE CONCEPT.

ESSY ASSAY

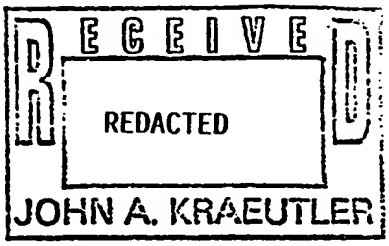
SCALE:	APPROVED BY:	DRAWN BY NWM
DATE: REDACTED		REVISED
CARTER WALLACE, CRANBURY NJ		
		DRAWING NUMBER



EASY ASSAY

SCALE:	APPROVED BY:	DRAWN BY NWM
DATE: REDACTED		REVISED
CARTER WALLACE, CRANBURY NJ		
		DRAWING NUMBER

EXHIBIT J



MEMORANDUM

TO: Distribution

FROM: Ms. M. Goddard

DATE: REDACTED

CONFIDENTIAL
ATTORNEY'S EYES ONLY

SUBJECT: GOLD SOL MEMBRANE MEETING - REDACTED

Attendees: C. Ageis
D. Charlton
M. Goddard
N. Miller
N. Tabolinsky
M. Verbanic

Prototype Development Status: Platform Concept

1. An order for 300 units from an aluminum tool has been placed with Delvco Industries, located in eastern Pennsylvania. This vendor is capable of form, fill, and seal operations.

2. Prototypes are due the week of REDACTED

3. R&D committed to provide additional dimensions to Neil Miller by REDACTED to keep this project moving.

4. Wettability of some urines raised the question of needing an overfilter, but issues such as this will be addressed with prototype data.

5. Gold conjugate has reached one month of stability at 4°C in liquid form, with no change to date in lyophilized stability.

6. The latex has shown two weeks of stability in foil with dessicant at 50°C; room temperature is "still going" after two weeks.

7. Attempts were made at lyophilizing the conjugate on the membrane, but feasibility is not yet established.

8. Work has continued on conjugate reconstitution with urine (for OTC use).

"Chromatograph" Direct Application Concept Status

1. Using b108 and 30nm gold, samples of the test were shown to function as follows:

   a. Lyophilize gold in tube
   b. Add urine
   c. Place membrane/stick into tube
   d. Wait 2-5 minutes and read.

2. The concept would incorporate die-cut plastics instead of molds and would expose reading "bars" which would be pre-coated with latex using a jet-spray process.

3. N. Miller agreed to make a "clicker die" for R&D use once given appropriate dimensions and recommended materials.

Related Issues

1. R&D has begun steps to scale up b105 conjugate for LH kit lyophilization.

2. Background color in negatives could pose a problem for domestic and international markets; however, the extent of this color cannot be assessed until prototypes are available.

A follow-up meeting will take place on REDACTED at 1:30 p.m. in the Wampole Conference Room in Cranbury.

MG:jlr

Distribution

C. Agius
R. Barrish
J. Bridgen
D. Charlton
A. Chizamadia
J. Dekis
P. Freidenreich
R. Herzog
A. Howell
J. Iltis
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S. Kolakowsky
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J. Kraeutler
R. McPartland
N. Miller
B. Packer
S. Riggio
B. Shears
N. Tabolinsky
C. Utz
M. Verbanic
W. Wallen

EXHIBIT K

OFFICE MEMORANDUM

TO: L. R. ISBRANDT

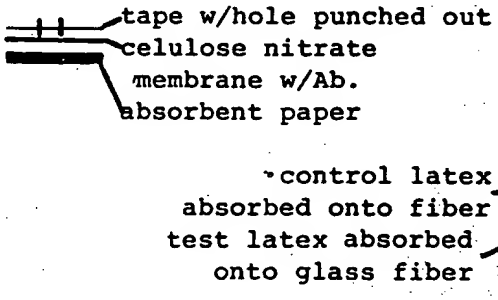
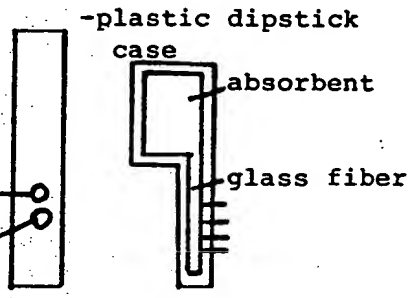
FROM: D. E. CHARLTON

DATE: REDACTED

SUBJECT: NEW HORIZONS VS. EASY ASSAY

cc: K. Clarke
S. Friedman
A. Howell
J. D. Mack
D. W. McLain
G. R. Pflug
C. R. Stafford

Wampole and Carter-Wallace International are currently negotiating a licensing and R&D agreement with New Horizons to use the colloidal gold-membrane format for pregnancy and strep-A test kits. The issue has arisen whether Carter R&D's Easy Assay format falls under the licensing agreement. As per the attached memo, the New Horizons test, as presented to Wampole, was not a feasible product; and as such, the recommendation was made not to proceed with New Horizons. Easy Assay in the colloidal gold format was made possible by the Wampole development of gold conjugates using B108 from Columbia and by using our 151-antibody coupled to latex. A comparison of the New Horizons test and Easy Assay is presented below.

Parameter	New Horizons	Easy Assay
Solid Phase	-Ab. attached to cellulose nitrate membrane	-Ab. attached to latex and dried onto glass fiber paper
Device Configuration		
Conjugate	-30nm gold particle w/? Ab. absorbed -lyophilized in dropper vial	-15nm gold particle w/ B-108 or B-105 Ab -lyophilized in 10x50mm test tube
Procedure	-add urine onto well, let absorb -add reconstituted gold conj. and let absorb -wash with buffer	-reconstitute gold conj. with urine -place dipstick into gold conj-urine mixture and let absorb for max. of 15 minutes
Steps	3	2
Test Time	2-3 minutes	1-15 minutes

Significant Differences Between Products

1. Different solid phase - capture system.
2. Different configuration.
3. Different size gold particle and antibody.
4. Different procedure.
5. Less steps.
6. Different test times.

It is our opinion that since both formats are significantly different in procedure and configuration and since prior art for using gold conjugates has existed for a number of years, the Carter Products Program does not fall under the licensing agreement with New Horizons.

David E. Charlton

DEC:jad
Attachment

EXHIBIT L

OFFICE MEMORANDUM

TO: L. R. ISBRANDT

cc: S. Friedman
A. Howell
G. R. Pflug

FROM: D. E. CHARLTON

DATE: REDACTED

SUBJECT: HYBRITECH PATENT

We have developed a substantial research effort in establishing and building a significant OTC diagnostic product line. In order to improve our products and continue to build our share of the market, we must use monoclonal antibodies as both the trap and conjugate. This combination gives us the speed, sensitivity and specificity required to provide the best possible products. Without a license from Hybritech to use monoclonal antibody combinations, the improved and future products are not possible, and as such our continual success in the OTC diagnostic business is at risk.

The following is a list of current and future projects utilizing monoclonal/monoclonal antibody combinations:

Project	Features	Improvements
ANSWER PLUS	- 30 minute test time - polyclonal Ab. trap - monoclonal Ab. conj. - 15% cross reaction w/LH	- <10 minute test time - monoclonal Ab. trap - monoclonal Ab. conj. - no cross reactivity with LH - better uniformity & availability of components
ANSWER OVULATION	- 30 minute test - polyclonal Ab. trap - monoclonal Ab. conj. - 100% cross reactive with hCG - uses more conjugate therefore higher cost	- <30 minute test - monoclonal Ab. trap - monoclonal Ab. conj. - ultimately no cross reactivity with hCG - better supply of components and better cost of goods
EASY ASSAY (hCG)	- <15 minutes - 1 step - monoclonal Ab. trap - monoclonal Ab. conj. - no cross reactivity with LH	
EASY ASSAY (LH)	- <15 minutes - 1 step - monoclonal Ab. trap - monoclonal Ab. conj.	

Future tests:
1. Infectious disease
2. Drugs of abuse
3. Other ovulation markers, i.e. progesterone & esterone
4. Etc.

D. Charlton

D. E. Charlton

DEC:jad

REGULAR UTILITY

Form PTO-436
Rev. 8/78)

SERIAL NUMBER	PATENT DATE	PATENT NUMBER
831013		

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
06/571,013	02/18/86	436	7	182	Doctor

WILLIAM L. BROWN, GRAYSLAKE, IL; JOHN M. CLEMENS, GURNEE, IL; SHARON M. DEVEREAUX, GURNEE, IL; JOHN G. HOFLER, INGLESIDE, IL; KEVIN M. KNIGGE, WHEELING, IL; SARAH E. SAFFORD, LIBERTYVILLE, IL- Vincent Tyus Libertyville Ill.

CONTINUING DATA********
VERIFIED THIS APPLN IS A CIP OF 06/784,416 10/04/85

B.E.D.

FOREIGN/PCT/APPLICATIONS********
VERIFIED

None filed

FOREIGN FILING LICENSE GRANTED 05/09/86

Foreign priority claimed 35 USC 119 conditions met	Verified and Acknowledged	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☐ yes ☒ no ☐ yes ☒ no	Examiner's Initials	→	IL	3	32	6	$ 696.00	4330

MARTIN L. KATZ
ABBOTT LABORATORIES (D-377)
14TH STREET & SHERIDAN ROAD
NORTH CHICAGO, IL 60064

Edward H. Gorman, Sr.

SOLID-PHASE ANALYTICAL DEVICE AND METHOD FOR USING SAME

U.S. DEPT. of COMM./Pat. & TM Office — PTO-436L (rev. 10-78)

PARTS OF APPLICATION FILED SEPARATELY

PREPARED FOR ISSUE

(Assistant Examiner) (Docket Clerk)

AT ALLOWANCE

DRWGS.	FIGURES DRWGS.	CLAIMS	CLASS	SUBCLASS

EXAMINED AND PASSED FOR ISSUE

(Primary Examiner) (Art Unit)

Estimate of printed pages		Issue fee due (est.)
Drawing(s)	Spec(s)	

Notice of allowance and issue fee due (est.)

Date mailed	Date paid

ATTENTION LABEL

labelled substance or reagent directed against the antibody (Amplification or Indirect immunoassay). Thereafter, unbound material is removed, e.g., by washing, and the device is contacted with an indicator substance which, in the presence of the "label" of the second reagent, produces a detectable response which is indicative of the presence and/or amount of the analyte in the sample. Such a detectable response can be read visually or instrumentally, and can advantageously be a color response, most desirably in the form of the visible appearance of a "+" or "-" sign to indicate the result of the assay, particularly if only positive or negative results, respectively, from the assay are necessary or desired. Alternatively, quantitative or semi-quantitative results can be obtained by visually or instrumentally reading the detectable response.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a side view in partial cross section of an analytical device in accordance with the present invention.

Fig. 2 is a top plan view of the device of Figure 1.

Figs. 3A, 3B and 3C are top plan views of a particularly preferred embodiment of the device of Fig. 1.

Fig. 4A, 4B and 4C are top plan views of an alternate embodiment of the device of Fig. 1.

Fig. 5 is a perspective view of the device of Fig. 1, showing the pre-filter removed from the body of the device.

DETAILED DESCRIPTION OF THE INVENTION

The novel material of the present invention, and devices produced therefrom, although applicable to many types of analysis, are especially advantageous when used in immunoassays, to improve conventional solid-phase immunoassay techniques for performing